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ZENILLIA LIBATRIX PANZER, A TACHINID PARASITE OF THE GYPSY MOTH AND THE BROWN-TAIL MOTH [1,2]

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INTRODUCTION

Early European rearings of the gypsy moth (*Porthetria dispar* L.) and the brown-tail moth (*Nygmia phaeorrhoea* Don.) for the purpose of obtaining natural enemies of these pests for liberation in the United States showed that *Zenillia libatrix* Panzer was a parasite of minor importance on both these insects. Later it was also reared in small numbers from the satin moth (*Stilpnotia salicis* L.). In 1928 a fairly large number of *Z. libatrix* were recovered from *P. dispar* reared at Vecs, Hungary, and study on the biology of the species was begun at the sublaboratory of the Bureau of Entomology, United States Department of Agriculture, in Budapest. This paper gives the results of the study and briefly discusses the attempts that have been made to establish the parasite in New England and the probability of its establishment.

REVIEW OF LITERATURE

References to *Zenillia libatrix* are fairly common. In 1908 Townsend (*17*)[4] briefly noted its habit of leaf oviposition and the fact that it is double-brooded. Pantel (*11, pp. 46, 48*) in 1910 placed it (under the genus *Myxexorista*) in his group II of leaf-ovipositing tachinids and figured the egg and an ovariole. Howard and Fiske (*8, pp. 90–91*) in 1911 and Burgess and Crossman (*5, p. 115*) in 1929 recorded it as a parasite of both the gypsy moth and the brown-tail moth and noted the number of flies liberated in the United States. In 1920 Baer (*1, p. 153*) gave a list of lepidopterous hosts attacked by *Z. libatrix*. Eidmann (*7*) in 1926 mentioned the fact that it is very polyphagous and its mode of gaining entrance into the host. Finally, in 1931, Brown (*4*) recorded it as a parasite of the satin moth and briefly indicated its life cycle.

DISTRIBUTION AND HOST RELATIONSHIPS

Zenillia libatrix is common throughout Europe. Rearings of gypsy and brown-tail moth material in connection with the work of the United States Bureau of Entomology show recoveries from

[1] Received for publication Oct. 2, 1933; issued March, 1934.

[2] This study was conducted under the direction of C. W. Collins, in charge of the Bureau of Entomology field laboratory, Melrose Highlands, Mass., at its European sublaboratory at Budapest, Hungary, during 1929, 1930, and 1931, and at Melrose Highlands in 1932.

[3] The writer expresses his gratitude to R. T. Webber for the use of his notes on *Zenillia libatrix* and for many helpful suggestions; to D. W. Farquhar for laboratory assistance at Melrose Highlands; to Ferencz Mihalyi for laboratory assistance at Budapest; and to W. F. Sellers, W. E. Ripper, R. C. Brown, and Josef Ujhelyi for help in making collections in Europe.

[4] Reference is made by number (italic) to Literature Cited, p. 113.

Spain, France, Germany, Austria, Czechoslovakia, Hungary, Italy, Yugoslavia, and Russia. Wainwright (*18*) records it from England, and Lundbeck (*9*) from Denmark, Sweden, and Finland. Howard and Fiske (*8, p. 302*), Baer (*1, p. 153*), and Brown (*4*) state that the species is principally a southern form, but the largest collections made of it in gypsy-moth work were in Hungary, while in rather extensive rearings in Spain, Portugal, and Morocco only a single specimen (from Spain) was reared.

Zenillia libatrix is very polyphagous. A list of hosts recorded in European literature follows. Those starred (*) are species from which, during this study, the parasite has also been reared from field-collected material.

LEPIDOPTEROUS HOSTS OF ZENILLIA LIBATRIX RECORDED IN EUROPEAN LITERATURE AND REARED FROM FIELD-COLLECTED MATERIAL IN HUNGARY

Abrostola asclepiadis Schiff. (*1, 3, 8, 9*)
Acronicta auricoma Fab. (*9*)
Brephos nothum Hbn. (*1, 3, 8, 9*)
Bupalus piniarius L. (*1, 7, 9*)
Dasychira pudibunda L. (*1, 3, 8, 9*)
Drepana cultraria Fab. (*18*)
**Euproctis chrysorrhoea* L. (=*Nygmia phaeorrhoea* Don.) (*1, 9*)
Larentia autumnalis Strom. (*1, 3, 8, 9*)
Liparis monacha L. (*2*)
Lozostege sticticalis L. (*10*)
**Lymantria* (=*Porthetria*) *dispar* L. (*1, 3, 8, 9*)
Malacosoma neustria L. (*1, 3, 8, 9*)
**Oxycesta geographica* Fab.[5]
Phlyctaenodes verticalis L. (*1, 9*)
Porthesia similis Fuess. (*1, 9*)
Pygaera anachoreta F. (*1, 9*)
**Pygaera pigra* Hufn. (*1, 3, 8, 9*)
Salebria marmorata Alph. (*13*)
**Stilpnotia salicis* L. (*4*)
Sylepta ruralis Sc. (*1, 9*)
Thaumetopoea processionea L. (*1, 3, 8, 9*)
Yponomeuta cognatella Hbn. (*9*)
Yponomeuta evonymella L. (*1, 3, 8, 9*)
Yponomeuta padella L. (*1, 3, 8, 9*)
Yponomeuta rorella Hbn. (*1, 9*)

Although *Pygaera pigra* was the only field-collected host in which *Zenillia libatrix* overwintered, the species also passed the winter successfully in *Oxycesta geographica*, *Calocasia coryli* L., and *Acronycta rumicis* L. attacked at the Budapest laboratory.

In the United States at the Melrose Highlands (Mass.) laboratory, D. W. Farquhar and R. M. Seeley, under the direction of R. T. Webber, successfully used the silkworm (*Bombyx mori* L.) to rear a summer generation of the flies, and they had a large number of native lepidopterous larvae, representing 23 species, attacked by *Zenillia libatrix* in an attempt to determine whether there was a suitable alternate host for the species in New England. They found that the parasite was capable of completing a summer generation in at least two species, *Euchaetias egle* Drury and *Melalopha inclusa* Hbn., and the writer, on returning to Melrose Highlands, successfully brought it through the winter on *M. inclusa*.

ECONOMIC IMPORTANCE

From 1906 to 1910 only 177 adults of *Zenillia libatrix* were liberated in New England. They were obtained from brown-tail-moth caterpillars collected in Europe. In 1923 the Bureau of Entomology resumed the importation of enemies of the gypsy moth and the brown-tail moth from Europe, but since that time no heavy infestations of *Nygmia phaeorrhoea* have been investigated there and collections of these two species have been confined almost entirely to *Porthetria dispar*. Recoveries of *Z. libatrix* were negligible until 1927, when 407

[5] Not recorded in literature but reared during this study.

puparia were reared from the gypsy moth at Moscenica, Yugoslavia. In 1928, 1,690 puparia were obtained from 230,000 *P. dispar* collected at Vecs, Hungary. Since that time recoveries have been small, exceeding 100 only twice; 163 were reared from 330,000 *P. dispar* collected at Galgamacsa, Hungary, in 1931, and 109 were reared from 320,000 *P. dispar* collected at Jánk, Hungary, in 1930.

Collections of satin-moth larvae were made in Vienna, Austria, from 1928 to 1930. In 1928, 40 *Zenillia libatrix* were recovered from 50,000 *Stilpnotia salicis;* in 1929, 124 were recovered from 90,000 larvae; and in 1930, 26 were reared from 90,000 larvae.

In 1930 about 6,000 brown-tail-moth larvae were collected in Hungary, but no *Zenillia libatrix* were reared. In 1931 no puparia of this species were reared from 1,500 *Nygmia phaeorrhoea* collected near Budapest, Hungary, but 22 puparia were reared from about 1,000 larvae collected at Oberpullendorf, Austria. In 1932, 186 *Z. libatrix* were reared from 5,600 larvae collected at the same place.

This summary shows rather conclusively that *Zenillia libatrix* is usually of slight economic importance as a parasite of the gypsy moth, the brown-tail moth, or the satin moth. Nevertheless, the fact that a large number of puparia were recovered at Vecs, Hungary, in 1928 indicates that the species may become important under favorable conditions. At times it may also be a valuable parasite of other host species which it attacks. Pustovoit (*13*) has recorded a parasitization by *Z. libatrix* of 10.5 percent on the procession moth (*Thaumetopoea processionea* L.).

TECHNICAL DESCRIPTION

ADULT

The adult fly was originally described as *Musca libatrix* by Panzer (*12*) in 1798. The original Latin description is very brief, but Lundbeck (*9, pt. 7, pp. 338–339*) has written the excellent redescription and synonymy which follow. Lundbeck calls the visible segments 2–5; the present writer calls them 1–4. Lundbeck omits the last segment entirely (his fifth, the writer's fourth).

1. *Z. libatrix* Panz.

1798. Panz. Faun. Germ. LIV, 12 (*Musca*)—1824. Meig. Syst. Beschr. IV, 400, 281 (*Tachina*) et 1838. VII, 256, 46 (*Exorista*)—1844. Zett. Dipt. Scand. III, 1163, 164 (*Tachina*)—1862. Schin. F. A. I, 464 (*Exorista*)—1891. B. B. Denkschr. Akad. Wiss. Wien, LVIII, 333 (*Myxexorista*)—1907. Kat. paläarkt. Dipt. III, 278—1907. Villen. Wien. Ent. Zeitg. XXVI, 254, 42—1921. Baer, Zeitschr. f. angew. Ent. VII, 153—1924. Stein, Arch. f. Naturgesch. 90, 6, 77, 14 (*Exorista*)—*Tachina fauna* Meig. 1824. l c. IV, 393, 268 et 1830. VI, 368 et 1838. VII, 256, 34 (*Exorista*)—1862. Schin. F. A. I, 464 (*Exorista*)—1900. Villen. Bull. Soc. Ent. de Fr. 159, 11, 12 et 1907. Wien. Ent. Zeitg. XXVI, 248, 5—1907. Kat. paläarkt. Dipt. III, 278.

All brownish-yellow pruinose species. Male. Frons above a little narrower than the eye, somewhat protruding. Orbits much broader than frontal stripe, yellow or almost golden; cheeks yellow above, silvery whitish below; jowls grey, nearly one third of the height of the eye; frontal stripe narrow, widening downwards, velvet black or brownish black. Weak outer vertical bristles present. Frontal bristles descending fully to the end of second antennal joint, two uppermost reclinate. Vibrissae ascending fully to the middle, the upper ones small. Orbits and jowls with black hairs. Occiput yellowish grey, with yellowish hairs, and black hairs behind postocular bristles. Eyes pale-hairy. Antennae black,

third joint about four times as long as second; arista as long or fully as long as antennae, thickened in basal half. Palpi yellow. Thorax yellow pruinose, with four narrow black stripes, the median abbreviated behind, the lateral interrupted at the suture into two elongated spots; scutellum bright yellow. Thorax black-haired. Three sternopleural bristles. Abdomen quite yellowish or brownish yellow pruinose, basal segment a little darker; it is black-haired, with a pair of marginal bristles on second segment, a pair of discal and marginal on third and a pair of discal and a row of marginal on fourth segment. Legs black, a little greyish pruinose. Wings yellow at base and anterior margin, outwards slightly tinged; veins brown; first posterior cell narrowly open, ending near apex of wing; discal angle obtuse; apical cross-vein about straight. Squamulae yellow or deep yellow. Halteres yellow.

Female. Similar; frons a little broader, as broad as the eye.

Length 6.5–8 mm.

* * * * * * *

Remarks: The species is known to vary in colour, being sometimes greyish, but our specimens are all brownish yellow. On account of its varying it has been described under several names; to the above synonymy still can be added: *Tachina dolosa* Meig., *Exorista ancilla* Meig., *Myxexorista macrops* B. B., *M. grisella* B. B., *Zenillia perplexa* Pand., *Z. discerpta* Pand., *Z. fulva* Pand., of these *fauna* Meig., *ancilla* Meig., and *grisella* B. B. belong to the greyish variety.

IMMATURE STAGES

Egg

The egg (fig. 1, *A*) is microtype, about 0.15 mm wide and 0.20 mm long. It is ovoid in shape, and there is a distinct micropyle at the cephalic extremity. The thick upper surface of the chorion is rounded and heavily pigmented with conspicuous reticulations; the lower surface is thin, flat, and transparent. Beneath the chorion there is a thin but strong vitelline membrane enclosing the embryo. A thin layer of gelatinous material sticks the egg to the foliage and may be seen protruding slightly around the ventral margin of the egg.

First-Instar Larva

The larva (fig. 1, *B*) increases greatly in size during the first instar. A specimen removed from the egg is 0.23 mm long and 0.10 mm wide. The 5-day-old specimen figured is 0.45 mm long and 0.16 mm wide. Maximum growth attained in this instar is about 2 mm in length and 0.56 mm in width. The larva is cylindrical, tapering anteriorly and rounded posteriorly. It is composed of the pseudocephalon, bearing the antennal and sensorial organs, and 11 body segments. The pseudocepnalon is unarmed, but on all the other segments except the eleventh the colorless cuticle bears rows of minute spines. The first three anterior (thoracic) segments are completely encircled with spines anteriorly. On the fourth (first abdominal) segment the band of spines is broken in the pleural region. The fifth, sixth, and seventh segments have small groups of spines on their anterior margins in the ventral region. The eighth, ninth, and tenth segments have small anterior groups and also small posterior groups in the ventral region. The eleventh segment is unarmed. The arrangement of the spines in rows and their comparative sizes are indicated in the figure. Those on the anterior margins of the segments are directed backward, those on the posterior margins are directed forward. In a small, young larva the spines are very conspicuous, but as the larva grows they become widespread and less prominent.

The buccopharyngeal armature of the freshly emerged larva is shown in figure 1, *D*. It has no articulations. The median tooth is well developed and the basal lobes are very lightly pigmented. There is a pair of lateral plates at the anterior end of the median tooth, and a single salivary-gland plate ventrad of the basal region. When the salivary-gland plate is turned on its side, a pair of minute openings can be seen at the anterior extremity. In fully developed first-instar larvae the buccopharyngeal armature presents a different appearance on account of the further sclerotization of the basal lobes (fig. 1, *E*).

The larva is metapneustic. The two posterior spiracles open on the dorsopleural portion of the last abdominal segment. The spiracular chambers (fig. 1, *C*) are about twice as long as broad.

The only sensory organs observed are the minute antennal and maxillary organs on each side of the mouth opening. The antennal organs are circular in outline

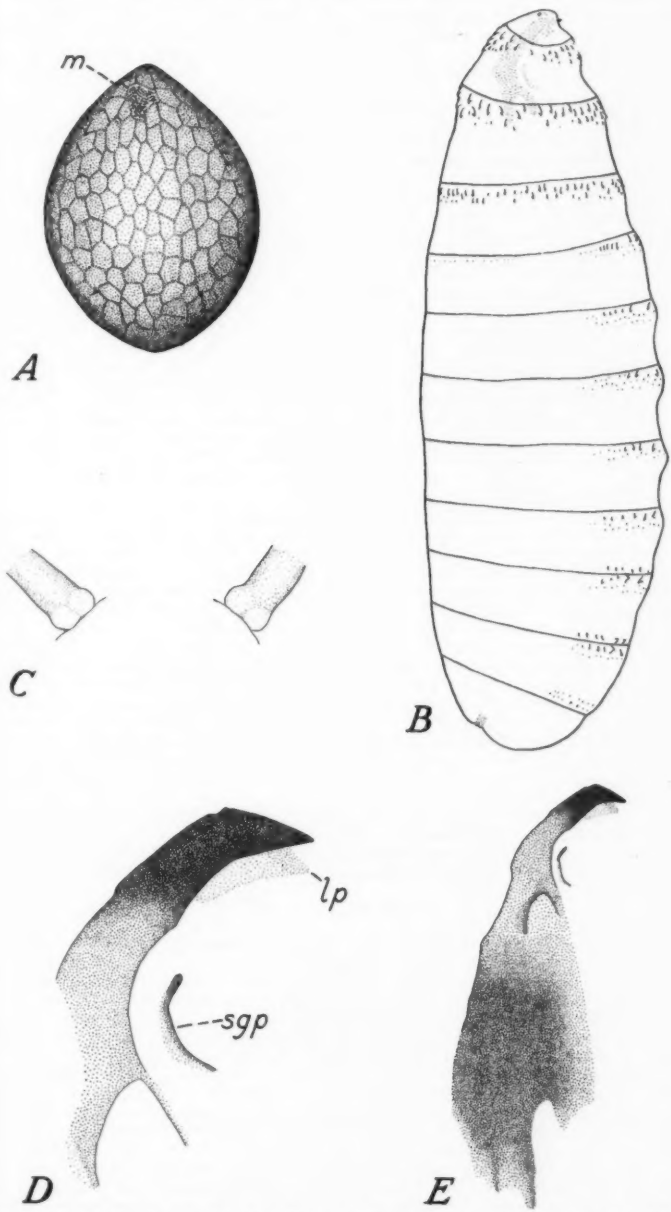

FIGURE 1.—*Zenillia libatrix*, egg and first larval instar: *A*, Egg, dorsal view, showing micropyle, *m* (× 220); *B*, larva, lateral aspect (× 250); *C*, posterior spiracular chambers (× 1,300); *D*, buccopharyngeal armature of freshly emerged larva, showing lateral plates, *lp*, and salivary-gland plate, *sgp* (× 1,000); *E*, buccopharyngeal armature of fully developed first-instar larva (×320).

and convexly protuberant, measuring 0.0025 mm in both height and breadth. Just below them are the maxillary organs, each composed of a group of minute points raised very slightly above the surface of the cuticle. Each group has 2 rather prominent points and above them 2 and below them 4 or 5 tiny points.

Second-Instar Larva

The second-instar larva (fig. 2, *A*) is about 3.3 mm long and 0.9 mm wide when first formed. A larva just about to molt to the third instar was found to be 6.25 mm long and 2 mm wide. It is more robust than the first-instar larva, and the posterior extremity is truncate rather than rounded. The cuticle is still colorless. Although the cuticular spines are much more numerous than in the first instar, they are smaller and weakly pigmented, and therefore far less conspicuous. The pseudocephalon is unarmed. There are completely encircling bands of spines on the anterior margins of the first three segments. Segments 4 to 10, inclusive, have only very small groups of spines on their anterior margins, and these are all in the ventral region. The posterior margins of these segments, on the contrary, are well armed. Segments 4 and 5 are not completely encircled, for there are no spines in the dorsal region. Segments 6 to 10, however, have completely encircling bands on their posterior margins. Segment 11 has no spines on the anterior margin, but on the posterior margin there are from 15 to 20 rows. Below the spiracles of this segment there are several rows of spines directed away from the spiracles in a dorsoventral direction. The other spines are directed anteriorly. Figure 2, *A*, shows the arrangement of the spines, but there is considerable variation in the number of rows in different specimens. The spines on the anterior margins of the segments are pointed backward and those on the posterior border are pointed forward, as in the first instar.

In this instar the buccopharyngeal armature (fig. 2, *B*) terminates in a pair of lateral hooks. There are no articulations. The dorsal wing of the ventral plate is much longer than the ventral wing, but there is no progressive sclerotization of the basal wings as in the first instar. At the base of the lateral hooks there is a small sclerite, the hypopharyngeal plate, and dorsad of it the epipharyngeal plate. These sclerites are essentially the same in the second and third instars. They will be described in connection with the description of the third-instar larva.

The second-instar larva is amphipneustic. The anterior pair of spiracles (fig. 2, *C*) present two minute papillae, which open on the posterior border of the first body segment. There are no pigmented spiracular chambers connecting them with the tracheae, although the faint suggestion of an unpigmented tube may be found in prepared specimens. The posterior pair of spiracles (fig. 2, *D*) are well developed. Each has two papillae and often one of them is branched, as in the figure. The heavy spiracular chambers are about one and one half times as long as broad. The scar formed from the first-instar spiracles is prominent at the base of the spiracular openings.

The sensorial organs on the pseudocephalon are practically the same as in the first instar. The only difference is that they are raised a little higher. In this instar 4 pairs of small sensorial organs, having the form of fingerlike projections, were noted on the last abdominal segment, 2 pairs above the spiracles and 2 below.

Third-Instar Larva

The third-instar larva (fig. 3, *A*) has the same form as the preceding instar. It is much larger, measuring 7.5 mm long and 2.5 mm wide to 11 mm long and 3.5 mm wide. The cuticle is colorless and transparent and armed as before with tiny spines, but in this instar the spines are relatively so small that it is hard to see them. The pseudocephalon is armed, bearing 3 or 4 short rows of spines on each side of the mouth opening. Segments 1 to 9, inclusive, have completely encircling bands of spines on their anterior margins. On segment 10 this band is incomplete, there being no spines in the dorsal region. Segment 11 has no spines on its anterior margin. The anterior bands on the first three segments are of nearly uniform width throughout, but the bands on the abdominal segments are much wider on the venter where they cover the pseudopodia. Proceeding posteriorly there is a diminution of spines on the dorsal region on the anterior margins of the segments. The first three segments have no spines on their posterior borders. Segment 4 has a few, and the number on each segment increases proceeding posteriorly. On the posterior margins of segments 7 to 11 there are completely encircling bands of spines. On segment 11 there may be as many as 20 rows directed anteriorly, and several rows below the spiracles have a dorsoventral

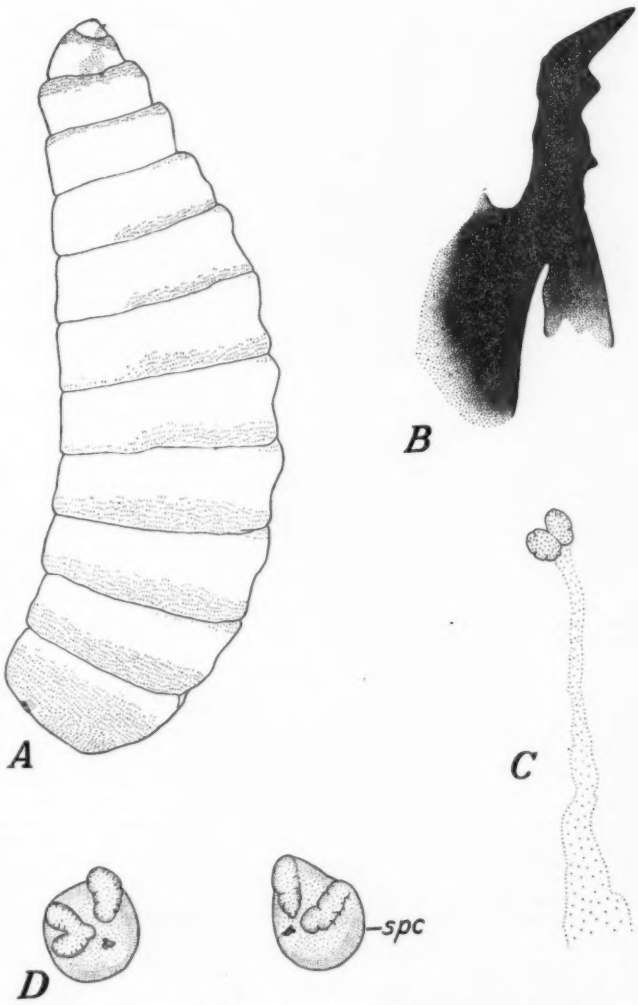

FIGURE 2.—*Zenillia libatrix*, second larval instar; *A*, Larva, lateral aspect (× 21); *B*, buccopharyngeal armature (× 160); *C*, anterior spiracles (× 600); *D*, posterior spiracles, showing spiracular chambers, *spc* (× 200).

direction. Figure 3, *A*, shows the disposition of the spines, but they are actually by no means as prominent as here indicated. The spines on the anterior margins of the segments point backward, and those on the posterior margins point forward as before.

The buccopharyngeal armature (fig. 3, *B*) is quite different from that of the second-instar larva. The lateral hooks are much straighter, there is an articulation between the intermediate region and the basal region, the dorsoanterior angle of the dorsal wing of the basal plate is strongly produced anteriorly, and there is a weakly sclerotized connection between it and the lower portion of the lateral hook. In many specimens a small opening is seen in the lateral hooks at the base of the ventral projection. It is believed that this is formed by the cells which generate the anterior hooks, as shown by Thompson (*16, p. 39, pl. IX, fig. 23*) in the larva of *Miltogramma punctatum* Meig., for in freshly molted third-instar larvae this opening is quite large. In many specimens the ventral projection of the lateral hooks seems to have a well-developed spine at its apex. This spine, though, is actually attached to the cuticle. Just above the intermediate region between the lateral hooks, the hypopharyngeal plate (fig. 3, *E*) may be distinguished. This is a small, irregularly shaped sclerite having two unsclerotized areas in the center of which are minute sensoria. The hypopharyngeal plate is attached basally to the intermediate region and anteriorly to the labium. The labium is a tough membrane armed with a varying number of well-developed spines. Just dorsad of the hypopharyngeal plate is the minute epipharyngeal plate (fig. 3, *F*). This plate varies considerably in shape. Often it is deeply emarginate. It has several unsclerotized areas bearing sensoria, as indicated in the figure.

The larva is amphipneustic in the third instar. The anterior spiracles (fig. 3, *C*) are well developed; they usually bear two papillae, and one specimen has been observed with three. The spiracular chamber is broad, and it widens abruptly into a cap fitting over the trachea. The posterior spiracles (fig. 3, *D*) are large, heavily sclerotized, and strongly raised. They are almost half as high as broad. Each spiracular plate has three respiratory slits. The molting scar is rather inconspicuous. The specimen figured was taken from a fairly young third-instar larva in order to show the respiratory slits well, but in an older larva almost the entire surface of the spiracle, with the exception of the slits and a small area around the molting scar, is pigmented to a deep black color.

The sensory organs on the pseudocephalon, antennal and maxillary, as well as the four pairs of fingerlike sensory organs on the eleventh segment, are the same as in the second instar, but they are even more prominently raised from the surrounding cuticle. No other sensory organs have been distinguished.

The Puparium

The puparia average about 8 mm long and 3 mm wide at the center. They are dull red. Both the anterior and posterior ends of the puparium are rounded. The posterior spiracles are situated a little above the longitudinal axis and just anterior to the apex. The spiracles are shiny black and raised almost half as high as their width. The anal opening is on the under side on the anterior margin of the eleventh segment. The anterior spiracles are very small but distinct.

The pupal respiratory apparatus (fig. 3, *G*) is of the reduced type, the prothoracic cornicles being absent. The tubes that usually connect them with the internal spiracles are present, and there is a small, slightly roughened, yellowish area in the cuticle of the prepupa where the apex of this tube comes in contact with the puparial shell. The internal spiracles are well developed. They bear about 200 respiratory papillae arranged along 5 or 6 radiating branches.

BIOLOGY AND HABITS

SEASONAL HISTORY

Zenillia libatrix passes the winter as a first-instar or second-instar larva within the host pupa and completes its development early in the spring. In the laboratory the first flies appeared April 6, 1928, May 18, 1929, and May 16, 1931. An adult male was taken in the field at Vecs, Hungary, on May 18, 1929.

The first adults of *Zenillia libatrix*, therefore, attack *Porthetria dispar* or some other host larva from about the middle of May until

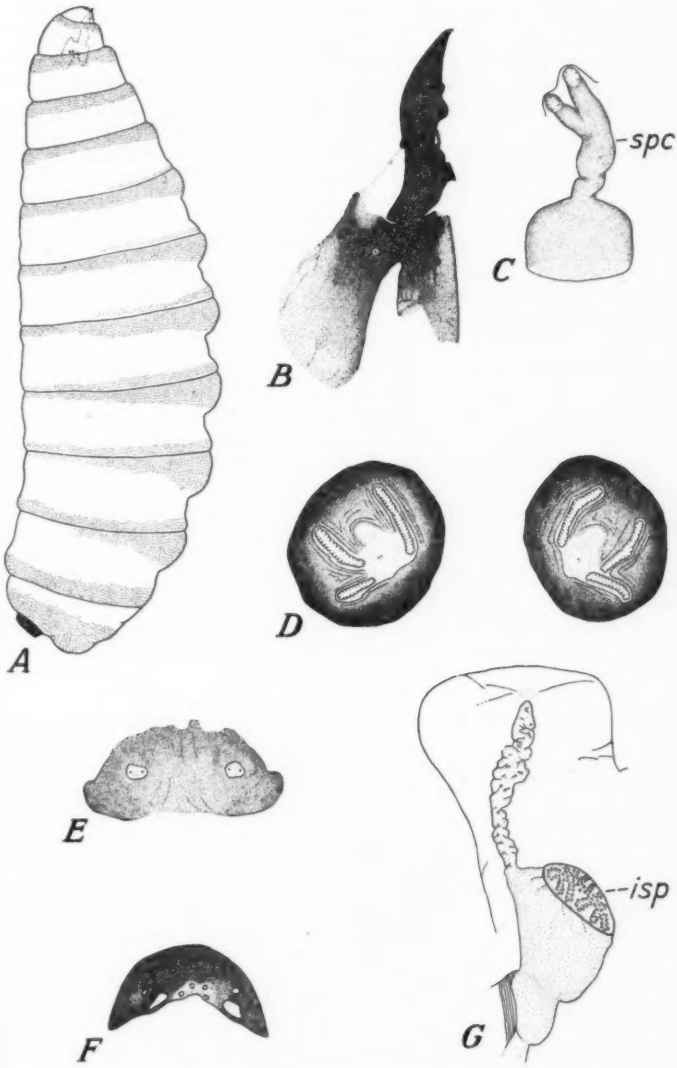

FIGURE 3.—*Zenillia libatrix*, third larval instar and pupa: *A*, Larva, lateral aspect (× 13); *B*, buccopharyngeal armature (× 75); *C*, anterior spiracles, showing spiracular chambers, *spc* (× 160); *D*, posterior spiracles, dorsal view (× 60); *E*, hypopharyngeal plate (× 425); *F*, epipharyngeal plate (× 500); *G* pupal respiratory apparatus, showing internal spiracle, *isp* (× 66).

the first of June or later. Puparia of *Z. libatrix* were recovered from field-collected *P. dispar* between June 22 and July 20 in Hungary. Adults issued between July 6 and August 6 in the laboratory, but probably somewhat later in the cooler forest. It is questionable whether another complete generation of *Z. libatrix* develops in nature. There is probably time, for a second summer generation is easily reared in the laboratory. Nevertheless, it seems doubtful, because of the scarcity of host material late in July and early in August and because flies issuing at that time could live long enough to attack larvae in which their progeny would overwinter. One female of *Z. libatrix* was taken at Vecs on August 25, 1928. The only field-collected larvae in which this parasite overwintered were *Pygaera pigra* Hufn. collected on October 6 and 10 near Budapest, Hungary.

In Hungary, therefore, *Zenillia libatrix* has at least two generations a year and possibly a partial third, while farther south it may have three regularly.

LENGTH OF LIFE

Zenillia libatrix adults live a considerable time under laboratory conditions. As many as five flies were held in glass-covered wooden boxes 5.9 by 7.9 by 3.9 inches (15 by 20 by 10 cm). They were fed lump sugar and honey solution (1 part of honey to 5 parts of water) held on sponges. When the flies were not being used, they were kept in a dark, cool place.

The average length of life of 18 mated females was 49.7 days, with a minimum of 27 and a maximum of 65 days; 21 males averaged 29.4 days, with a minimum of 8 and a maximum of 49 days; and 6 unmated females averaged 47.1 days, with a minimum of 34 and a maximum of 56 days. Of 4 males and 2 females issuing on July 12 which were given neither food nor water, 2 of the males lived 4 days, the other 2 males lived 5 days, and both females lived 5 days.

MATING

Zenillia libatrix adults mate readily in cloth-covered cages held in the light, but not in the direct sunlight. They prefer the morning hours. Temperatures from 64.4° to 77° F. (18° to 25° C.) are favorable. The male flies are particularly attracted by flying females. The average time in coitus, for 16 pairs, was 85 minutes, with a minimum of 35 and a maximum of 130. Although females up to 3 or 4 days old mate fairly readily, freshly emerged females and males from 2 to several days old mate best.

Female flies never mated more than once, but experimentation indicated that 1 male can fertilize about 8 females. One hundred eggs were examined from each of 8 females fertilized by a single male, and at least 93 percent of the eggs from each female were fertile. A similar examination of the eggs of 8 females fertilized by another male showed that the eggs of each of the first 7 females were at least 92 percent fertile, while the eighth female had only 3 percent of her eggs fertilized.

PREOVIPOSITION PERIOD AND REPRODUCTIVE ORGANS

The reproductive organs of a freshly emerged *Zenillia libatrix* female are shown in figure 4, *A*. Each ovary consists of a number of ovarioles (fig. 4, *B*). Dissections showed that in some small flies there were only 61, while in one very large fly there were 92 in one

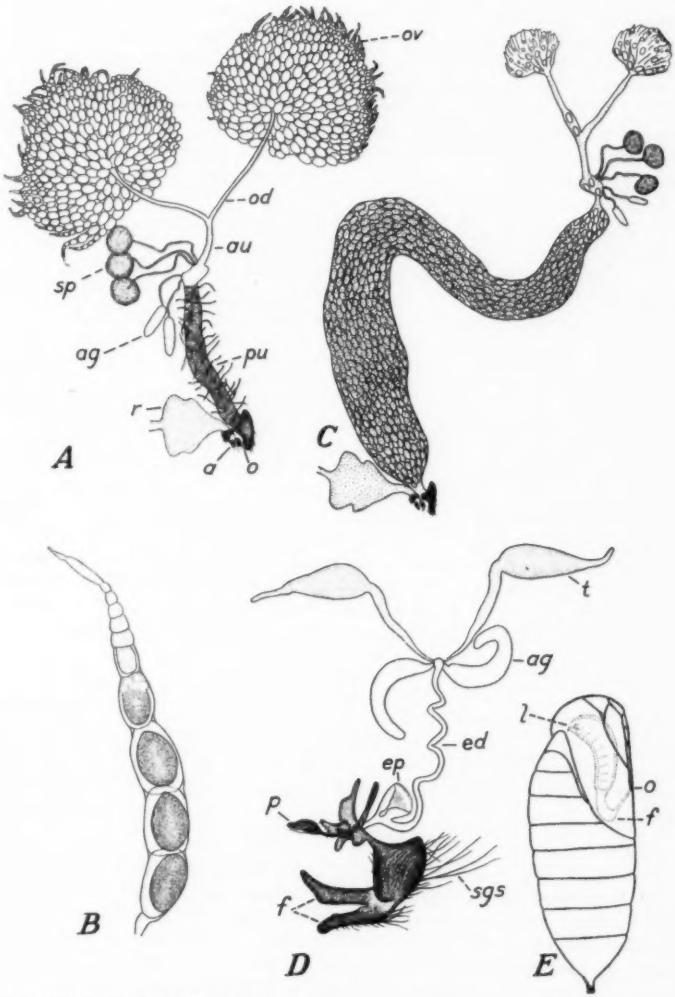

FIGURE 4.—*Zenillia libatrix*: *A*, Reproductive organs of freshly emerged unmated female; *ov*, ovary; *od* oviduct; *au*, anterior uterus; *sp*, spermatheca; *ag*, accessory gland; *pu*, posterior uterus; *r*, rectum; *a*, anus; *o*, ovipositor (× 13). *B*, Ovariole (× 38). *C*, Reproductive organs of mated ovipositing female (× 10). *D*, Male reproductive organs: *t*, testis; *ag*, accessory gland; *ed*, ejaculatory duct; *ep*, ejaculatory pump; *sgs*, second genital segment; *p*, penis; *f*, forceps (× 17). *E*, Host pupa with *Z. libatrix* larva in situ; *o*, opening to outside air, *f*, tegumental funnel; *l*, parasite larva (× 2).

ovary and 107 in the other. In a freshly emerged female about 10 developing eggs can be seen in each ovariole. If the fly is held several days before mating, all the eggs that will develop can be distinguished. Dissection showed that 14 is the usual number in an ovariole.

After the fly is mated, the eggs develop rapidly. The mature eggs are pushed into the oviducts and pass the spermathecal opening, where they are fertilized, and from there descend to the posterior uterus. The posterior uterus, which is quite short in the unmated female, enlarges enormously as the eggs descend into it until it forms a complete coil, taking up most of the fly's abdomen. Figure 4, *C*, shows the organs of a fertilized female extended in order to show the various parts.

Oviposition does not begin until practically all the eggs are in the posterior uterus. Six females were mated and isolated on July 15. One of them started ovipositing in 8 days, 3 in 9, and 2 in 10 days; but it was not until a few days later that they oviposited eagerly.

The male reproductive organs are shown in figure 4, *D*.

OVIPOSITION

The fact that *Zenillia libatrix* females deposit their eggs on foliage has already been noted. The object of this peculiar habit is to effect entrance into a host larva with the eaten foliage. In confinement the flies oviposit readily on almost any type of leaf, usually along the leaf margin. The fly bends the abdomen forward between its legs and deposits the egg by touching the surface of the leaf. A bit of gelatinous substance on the ventral surface of the egg sticks it to the leaf. The presence of a host larva undoubtedly stimulates oviposition, and if the edges of the leaf are cut the flies will oviposit along these cuts very readily, whether host larvae are present or not. Evidently the flies can sense that a feeding larva is probably responsible for fresh cuts in a leaf.

REPRODUCTIVE CAPACITY

Dissection of several female flies showed that their reproductive capacity varied considerably with the size of the fly. One very small fly had only 820 eggs, while the greatest number found was 2,439. The average number of eggs from 10 flies reared from *Porthetria dispar* was 1,820.

VIABILITY OF EGGS

When *Zenillia libatrix* eggs are laid, the embryos are completely developed, and upon examination distinct movement can be seen. Observations were made to determine how long the eggs that are not eaten remain viable after deposition. Environmental factors affect the eggs so much that it seemed to be impossible to determine the percentage which lived a specific number of days or even their average length of life. Eggs laid on the upper surface of the leaf and exposed to sunlight lost their viability more quickly than those laid on the under surface of the leaf. It was therefore decided simply to try to find the maximum number of days after deposition that eggs would remain so virile that host larvae eating them would become parasitized. For this purpose flies were induced to oviposit on the foliage of trees by confining them for several hours, in small cages, over a few of the leaves. The leaves were then left exposed on the

trees and on successive days were cut and fed to host larvae. In 1931, at Budapest, Hungary, when *Porthetria dispar* larvae were fed *Z. libatrix* eggs from 1 to 12 days old, larvae that ate eggs from 1 to 7 days old were killed by the parasite. In 1932, at Melrose Highlands, when larvae of *Bombyx mori* L. were fed eggs from 8 to 22 days old, the larvae that ate eggs from 8 to 15 days old produced *Z. libatrix*. It is therefore apparent that some of the eggs remain viable at least 15 days. Another fact brought out by these observations was that, although the eggs are scraped off the foliage rather easily when a drop of water is applied, they stick on very well during heavy rains.

MODE OF ENTRANCE INTO HOST

The following observations on the entrance of the parasite into its host were made for the writer by D. W. Farquhar:

By means of the binocular microscope, larvae of *Euchaetias egle* Drury were observed feeding on foliage bearing *Zenillia* eggs. The larvae assumed a position along the edge of the leaf, parallel to its axis, biting off areas of the margin with their laterally operating mandibles. Since the areas bitten off are considerably larger than the eggs, most of the latter are entirely engulfed. However, if the mandibles strike the eggs a glancing blow, the eggs are forced either into the mouth or back onto the leaf to be consumed later. If the mandibles strike the eggs a direct blow, the maggot is ejected from the egg and either enters the mouth of the host or adheres to the foliage until subsequently retaken. Although not observed in the laboratory, it is probable that some eggs or maggots, when struck by the jaws of the host caterpillar, drop to the ground and are lost.

An effort was made to locate the portion of the alimentary tract in which the eggs hatched. Dissection of a *Euchaetias egle* larva 5 minutes after it had fed on foliage bearing *Zenillia* eggs showed both maggots and unhatched eggs distributed from the anterior end of the alimentary tract to the large intestine. The maggots were crawling about in the lumen of the canal, none having penetrated the wall. Those eggs that had not hatched yielded maggots readily when lightly compressed with the forceps. An examination of the excrement of parasitized caterpillars showed many empty eggshells but no unhatched eggs. To determine whether the digestive fluids alone were the direct cause of hatching, the entire digestive tract of caterpillars was dissected out, dried externally to remove all blood, slit open, and eggs were introduced. A pronounced swelling of eggs ensued, owing to imbibition of the digestive juices, but none of them hatched.

From these experiments it appears that (1) hatching is not limited to any one part of the alimentary tract but occurs generally throughout its length, and (2) the digestive juices do not alone cause the eggs to hatch, but they induce a swelling of the eggs which renders them more susceptible to rupture due to the variations in pressure caused by motions of the digestive tract and of the caterpillar as a whole. It is probable that the absorption of the digestive fluids is followed by increased activity of the maggot, which assists in the hatching, particularly in the rupture of the vitelline membrane.

The writer also found that *Zenillia* eggs failed to hatch when immersed in fluids removed from the digestive tract of *Porthetria dispar*, but Severin, Severin, and Hartung (*15*) observed that the microtype eggs of *Chaetogaedia monticola* Bigot hatched very well when placed in the alkaline juices emitted from the mouths of several species of host larvae.

LARVAL DEVELOPMENT

The time required for *Zenillia libatrix* to complete its larval development depends more on the development of the host than on the parasite itself. The parasite larva hatches very soon after the egg is eaten and bores through the wall of the alimentary tract into the body cavity. It then enters one of the large abdominal muscles, the

silk glands, or occasionally a histoblast. Dissections have shown 46 in silk glands, 37 in muscle fibers, 2 in histoblasts, and 35 "floating free." Those found floating free were probably in muscle fibers that were ruptured by dissection. Larvae have been found in the silk gland 48 hours after the egg was eaten, and it is probable that they reached there some time before that.

The tiny first-instar larva remains in one of these three locations, developing slowly until the host larva starts to pupate. During this period it has no connection with the air and must obtain its supply of oxygen through its body wall or from ingested blood. When the host larva starts to pupate, the parasite larva migrates to the anterior portion of the forming pupa. At this time it starts to grow rapidly, and the basal lobes of the buccopharyngeal armature become sclerotized, as shown in figure 1, *E*. The parasite now forces a small opening in the host pupa between any of the ventral plates of the head sclerites. The irritation set up at this point results in an ingrowth from the host pupa, which rapidly turns dark brown and forms around the parasite larva in the shape of a funnel with the narrow end open to the outside air. This peculiar formation, which is common among the Tachinidae, assures the parasite of a constant supply of air. In *Zenillia* the funnel tube is long and narrow and sharply angled where it widens out (fig. 4, *E*). The wide part of the funnel rests against the inside of the host pupal shell. The rest of the parasite's larval life is completed rapidly. It molts to the second instar soon after the funnel is formed. As it grows the funnel also increases in size. After it molts to the third instar, it rapidly devours most of the contents of the host pupa and issues by cutting an opening between the abdominal segments. Occasionally it forms its puparium inside the host pupa, and the fly issues by breaking the pupal shell.

Because larvae of *Zenillia libatrix* do not begin to develop rapidly until the host larva pupates, the time required for development is variable. Often, when two host larvae are attacked on the same date, one will pupate within a few days while the other may require as long as 2 weeks or more. In such cases the *Zenillia* maggot in the host that pupated may issue and form its puparium while the *Zenillia* maggot in the host larva that did not pupate is still in the first instar. In laboratory rearings 2 *Porthetria dispar* pupae, the larvae of which were fed eggs on August 8, produced *Z. libatrix* puparia 15 and 16 days after attack, and 19 *Stilpnotia salicis* larvae that were fed eggs on August 2 pupated and produced 6 *Z. libatrix* puparia 19 days after attack. In most of the laboratory rearings, though, development was much slower. On August 6, 123 fourth-instar *P. dispar* larvae were attacked, and from them 103 *Zenillia* puparia and 11 *P. dispar* moths were reared. The first puparia were formed on September 1 and the last on September 17. Maximum formation of puparia occurred September 10 and 11, 35 and 36 days after attack.

Zenillia libatrix larvae overwinter in the first or second instar within the funnel formed in their host pupae. Most of the overwintering larvae are in the second instar, but evidently their development is sometimes arrested by cold temperatures before they molt to this instar.

Zenillia libatrix larvae are so tiny (0.23 mm long and 0.10 mm wide) when first hatched that Townsend (*17*) considered it probable that there were 4 larval instars in *Zenillia* and other tachinids laying microtype eggs, as compared with 3 in other tachinids. First-instar *Zenillia* larvae at all stages of development were therefore examined. By comparing the number and arrangement of the cuticular spines, and the size of the anal spiracles and spiracular chambers, it was definitely determined that *Zenillia* has only 3 larval instars. The enormous growth in the first instar is, nevertheless, very striking. Larvae only 0.23 mm long when hatched regularly increase to 10 times this size, or about 2 mm, without molting. Such an increase may, however, be more common than was formerly supposed. Cushman (*6*) found that the ichneumonid *Thersilochus conotracheli* Riley also increases enormously in size during the first instar. He does not give dimensions of freshly emerged larvae, but the egg is only 0.33 mm long and a full-grown first-instar larva is 2.00 mm long.

PERIOD SPENT AS A PUPARIUM

After issuing from the host pupa, the full-grown *Zenillia* maggot burrows into the ground a short distance and forms its puparium. No records of the time between the formation of the puparium and the issuance of the adult flies were made under natural conditions. Table 1 shows the time required when the puparia were held in the laboratory. Records were made in September.

TABLE 1.—*Time spent by Zenillia libatrix in the puparium when held in laboratory*

Time after formation of puparium (days)	Adult males issuing	Adult females issuing	Time after formation of puparium (days)	Adult males issuing	Adult females issuing
	Number	*Number*		*Number*	*Number*
11	4	1	15	0	19
12	7	2	16	0	4
13	18	1	17	0	1
14	16	15			

As is common among the Tachinidae, the majority of female *Zenillia* issued about 2 days later than the majority of the males. This is an important factor in mating, for the best mating is obtained between freshly emerged females and males from 1 to several days old.

LABORATORY REARING

Zenillia libatrix did not hibernate readily in laboratory-reared material. At Budapest and also at Melrose Highlands, Mass., a large number of host larvae, in which the species develops a summer generation, and which overwinter as pupae, were attacked late in September and early in October. A few of the parasite larvae hibernated, but the majority completed development and the flies issued so late that they could not possibly have found host larvae to parasitize.

Observations were made on the number of *Zenillia libatrix* that could complete development in a single *Porthetria dispar*. Sixty-three host larvae that ate *Zenillia* eggs pupated, and the pupae were isolated. Thirty-seven produced no parasites, 20 produced 1 puparium, 4 produced 2 puparia, 1 produced 3, and 1 produced 5 puparia.

At Melrose Highlands 8 puparia were recovered from a single pupa of *Bombyx mori*.

Fifty *Porthetria dispar* larvae that ate different numbers of eggs of *Zenillia libatrix* were isolated and reared for parasites. In this small number of examples there seemed to be no correlation between the number of *Zenillia libatrix* eggs eaten and the probability that a parasite would develop. One larva that ate 2 eggs pupated, and from these eggs 2 *Zenillia libatrix* completed development; but in a number of instances no parasite developed even when from 10 to 30 eggs were eaten. No superparasitism was detected in these specimens.

It was at first believed that, unless host larvae eating *Zenillia* eggs were fairly large, the eggs would be crushed. It was found, however, that a large percentage of *Zenillia libatrix* completed development in *Porthetria dispar* that were fed eggs when they were small third-instar larvae.

FACTORS LIMITING THE EFFECTIVENESS OF ZENILLIA LIBATRIX AS A PARASITE

The fact that *Zenillia libatrix* has never been reared in large numbers from *Porthetria dispar*, *Nygmia phaeorrhoea*, or *Stilpnotia salicis*, although some puparia are usually recovered from large-scale rearings of each of these hosts, indicates that there must be factors seriously limiting the effectiveness of the species as a parasite. It surely is not the fly's habit of ovipositing on leaves, for *Sturmia scutellata* R.D., one of the most effective of all *Porthetria dispar* parasites, gains entrance into the host in the same manner. Climatic conditions can also hardly be responsible, for *Zenillia* has a wide distribution. *Zenillia* may not be entirely suited to these hosts, but that also hardly seems likely, for when the eggs are eaten under laboratory conditions a fairly large percentage of attacked larvae produce the parasite. No natural enemies of *Zenillia libatrix* were observed, and in *Porthetria dispar* no maggots killed by phagocytes were noted.

A few facts have been noted in rearing work, however, which might be partly responsible for the parasite's low effectiveness. The fact that the species is very polyphagous would seem to limit its effectiveness on any one species. Since *Zenillia libatrix* is double-brooded, a great many individuals complete a generation in the fall after suitable host larvae have gone into hibernation. As these flies perish without reproducing, a small number of flies issue in the spring. Probably the most important limiting factor, though, is that the species must be severely handicapped by parasitic competitors. It requires a long period of development, and host larvae containing small *Zenillia* maggots might be attacked by other parasites, such as *Phorocera agilis* R.D., which would complete development and issue from the host larvae before *Zenillia libatrix* even molted to the second instar. It seems to be just as readily defeated in competition by *Sturmia scutellata*, which usually issues from the host pupa, for this species develops beyond the first instar in the host larva, and therefore when the host pupates it is considerably further developed than *Zenillia*. At Vecs, Hungary, so many puparia of *Zenillia libatrix* were recovered in 1928 that a fair recovery was expected in 1929. As a matter of fact, the species practically disappeared. Apparently it had overwintered successfully, for a male adult was taken in the field that spring. That summer parasitization by *Sturmia scutella* increased enormously.

PROBABILITY OF ESTABLISHMENT

From 1906 to 1910 only 177 adult *Zenillia libatrix* were liberated in New England. In 1927, 327 adults were liberated, in 1928, 1,004 adults, and from 1929 to 1932 only 129 adults were liberated. This is a small number, but, since laboratory work has indicated that the species can hibernate in at least one common native lepidopterous larva, it is quite possible that it has been able to survive. It has never been recovered from collections of *Porthetria dispar*, *Nygmia phaeorrhoea*, and *Stilpnotia salicis* in New England, but perhaps further collections will show that it is actually established.

SUMMARY

Zenillia libatrix is a leaf-ovipositing tachinid, common throughout Europe. It is a parasite of minor importance on the gypsy moth, brown-tail moth, and satin moth and attacks many other species of lepidopterous larvae. It has been liberated in New England but has not yet been recovered.

The various stages of the parasite have been described in detail.

A study of the life history of *Zenillia libatrix* has shown that it has two generations a year and possibly a partial third. The winter is spent as a first-instar or a second-instar larva within the host pupa. The first generation is completed on *Porthetria dispar* or some other host available during May, June, and early July. A second generation may be completed in August, but probably larvae of the second generation hibernate. The parasite has been reared in the spring from larvae of *Pygaera pigra* collected in the field in October.

The adult flies live and mate well in confinement. The females oviposit readily on leaves that have been fed upon or cut. The average number of eggs produced by one female is about 1,800. The eggs may remain viable for as long as 15 days after oviposition. The eggs are eaten by host larvae as they feed upon foliage, and the tiny *Zenillia* larvae hatch and bore their way through the alimentary tract. They enter the silk gland, one of the abdominal muscles, or a histoblast and develop very slowly until the host pupates. They then migrate to the anterior portion of the host pupa, form an integumental breathing funnel, and rapidly finish development. The full-grown larva issues from the host pupa and forms its puparium in the ground. The male usually spends 13 or 14 days in the puparium, the female 14 or 15 days.

As many as 5 *Zenillia* larvae may complete development in a single specimen of *P. dispar*, although usually only 1 parasite issues from a host.

The effectiveness of the parasite seems to be limited by its polyphagous habits, the fact that it is double-brooded, and its slow larval development, which makes it a poor competitor of other larval parasites.

It may be established in the United States, for although only small numbers have been liberated, it has been found to overwinter in a common native species, *Melalopha inclusa*, attacked at the laboratory.

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DEVELOPMENT OF A STANDARD CAGE METHOD FOR TESTING THE EFFECTIVENESS OF STOMACH-POISON INSECTICIDES ON THE JAPANESE BEETLE [1]

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INTRODUCTION

It is difficult to determine definitely the effectiveness of stomach-poison insecticides on the adult Japanese beetle (*Popillia japonica* Newm.) in the field because of the habits of the insect. The beetle is a strong flier, moving intermittently from one plant to another. A nucleus of beetles on a plant attracts other beetles flying in the vicinity. This insect is generally repelled from foliage on which there is a spray deposit (*4*, *6*, *8*, *13*).[3] When beetles do alight on foliage that has been sprayed with a stomach-poison insecticide, some leave without feeding, some consume a sublethal dose, some consume a fatal dose and fly elsewhere to die, and a relatively small number die on or near the plant. It was early recognized that the beetle would have to be confined and kept under observation before the insecticidal value of a material for it could be determined.

Campbell (*1*), after experimenting with silkworms, proposed a method for determining toxicity based on the introduction of a known quantity of a poison into the body of a feeding mandibulate insect by placing a drop of known concentration in its path and permitting it to imbibe the liquid. This method was tried without success with the Japanese beetle. It did not imbibe readily or completely a drop of liquid placed in its path on a leaf but usually walked through the drop, scattering the liquid over the surface of the leaf. Attempts at forced feeding through the mouth also proved futile because of regurgitation by the insect.

Van Leeuwen (*12*) confined Japanese beetles in individual cages with foliage that was coated with a known quantity of lead arsenate. By measuring the area of leaf eaten he was able to calculate the quantity of poison taken into the alimentary tract. This work indicated that the sandwich method of Campbell and Filmer (*3*) and Campbell (*2*), which overcomes any errors introduced by the material being brushed off the foliage or clinging to the body of the insect and which has been used successfully by Richardson and Haas (*11*) with larvae of the Colorado potato beetle, might be used to determine the smallest quantity of a compound necessary to kill the Japanese beetle.

There are, however, several difficulties connected with testing stomach-poison insecticides against the Japanese beetle in this manner: (1) As each feeding individual must be observed continuously, few materials can be tested at one time and little can be accomplished in the short season that the Japanese beetle is available; (2) the beetle

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[3] Reference is made by number (italic) to Literature Cited, p. 130.

is a gregarious insect and reacts abnormally when isolated in individual cages; (3) the beetle is an intermittent feeder, moving from one part of the foliage to another and making several punctures which are usually so small that in order to measure them accurately it is necessary to project an enlarged image of the eaten portion of the leaf.

The method by which several insects are confined in each cage with treated foliage and the mortality is determined at intervals seemed to be best adapted for work with a gregarious insect such as the Japanese beetle. As beetles survive in these cages from one to several days, it is not necessary to keep them under constant observation. The investigator is thus able to conduct many tests during the short period in the summer when the Japanese beetle is available for experimentation. This procedure measures the effectiveness or noneffectiveness of a material, but it does not, of course, measure its actual toxicity. The toxicity of a stomach-poison insecticide should be based on the actual weight of the material in the alimentary tract which is fatal to the insect.

TESTING MATERIALS IN WIRE CAGES IN AN OPEN INSECTARY

The procedure that has been used for the last 10 years at the Japanese beetle laboratory has been to confine beetles in a wire cage (fig. 1)[4] in an open insectary with foliage sprayed with a material of known concentration and to compare their death rate with that of beetles confined under the same conditions with untreated foliage. Certain phases of these studies have been published by Moore and Campbell (*9*), Van Leeuwen (*12*), and Fleming (*5*). The results obtained in successive tests with a material by this procedure varied considerably because of differences in the temperature and the relative humidity in the insectary.

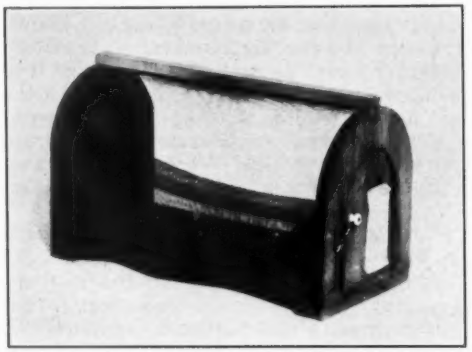

FIGURE 1.—Wire cage used for testing the effect of stomach-poison insecticides on Japanese beetles.

PROCEDURE

A series of experiments were conducted to determine the variations in the death rate of Japanese beetles when confined in wire cages and to correlate these variations as far as possible with the environmental factors of temperature and humidity in the insectary. Fifty-one tests were made at daily intervals from July 7 to August 26, 1926, which is practically the period when the beetle can be found readily in the field in southern New Jersey. In each test 100 beetles were

[4] This cage, which was devised by J. J. Davis, formerly in charge of the Japanese beetle laboratory, is 24 inches long, 12 inches wide, and 14 inches high. The bottom and the ends are of wood. The sides and the top are covered with 16-mesh wire cloth. A hinged door at one end permits the introduction of insects and plants.

placed in two cages with potted plants of smartweed (*Polygonum pennsylvanicum*) that had been sprayed with lead arsenate [5] at a concentration of 5 pounds to 50 gallons of water, 100 beetles were confined in two cages with unsprayed plants of this variety, and the same number were confined in two cages without food. The temperature and relative humidity were recorded hourly between 7 a.m. and 6 p.m., and the beetles alive in each cage were counted at 24-hour intervals for 5 days. At the end of the season the maximum, minimum, and average percentages of mortality of the beetle, when confined with sprayed foliage and when confined without food, were determined by the following formula:

$$\frac{\text{Number alive with unsprayed foliage} - \text{number alive with sprayed foliage or without food}}{\text{Number alive with unsprayed foliage}} \times 100 = \text{percent mortality}$$

ACCURACY OF RESULTS WHEN BEETLES ARE COLLECTED IN THE FIELD

In order to have individuals of known age and history, an attempt was made to rear beetles for these tests. A sufficient supply could not be obtained in this manner, so beetles were collected daily, as required, from unsprayed foliage in orchards and fields. It was suspected, because of their feeding habits, that many of these beetles had also fed on sprayed foliage. An experiment was conducted to determine the possible error introduced from this source. At intervals during the summer, beetles were collected from unsprayed foliage on golf courses and farms. Other beetles were reared in the insectary in order to have a group of known history for comparison. One hundred beetles from each group were placed in two cages in the insectary with unsprayed smartweed, and their survival was determined over a period of 5 days. At the end of this period the beetles were digested with arsenic-free nitric and sulphuric acids to destroy the organic matter, and the quantity of arsenic was determined by the standard Gutzeit method.[6] Since arsenic oxide is 32.1 percent of this lead arsenate, the quantity of lead arsenate equivalent to the oxide was found by multiplying by 3.125. Table 1 shows the survival of these beetles and the average quantity of arsenic oxide found per beetle.

TABLE 1.—*The arsenic content and the survival of adult Japanese beetles taken on unsprayed foliage in the field, and of beetles reared in the insectary*

Source of beetles	Beetles in test	Quantity of arsenic per beetle		Survival of beetles				
		Arsenic oxide (As_2O_3)	Equivalent lead arsenate	First day	Second day	Third day	Fourth day	Fifth day
	Number	*Milligram*	*Milligram*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Golf course	500	0.000061	0.000190	99.2	96.6	95.4	94.8	94.2
Cornfield	100	.000150	.000469	100.0	100.0	100.0	100.0	100.0
Grape vine	100	.000156	.000488	100.0	96.0	94.0	94.0	92.0
Hedgerow	300	.000149	.000466	100.0	95.3	93.0	92.3	91.6
Apple orchard	1,400	.000154	.000481	99.3	97.9	96.2	94.0	90.3
Reared in the insectary	300	.000047	.000147	100.0	100.0	96.3	95.0	93.3

[5] In the tests reported in this paper, ordinary commercial acid lead arsenic containing 32.1 percent As_2O_3 was used.

[6] R. E. Hulse, formerly agent, U.S. Department of Agriculture, made these analyses.

All the beetles, regardless of the source, contained some arsenic. The beetles collected on the golf course had probably emerged from the ground only a short time prior to capture, because their arsenic content was practically the same as that of the beetles reared in the insectary. The arsenic content of the beetles obtained on the farms was approximately three times that of the beetles reared in the insectary, indicating that some had fed on sprayed foliage. The average arsenic content of the beetles captured in the field ranged from 0.000061 to 0.000156 mg of arsenic oxide per beetle, which is equivalent to 0.000190 and 0.000488 mg, respectively, of lead arsenate. Van Leeuwen (*12*) has estimated that a dose of lead arsenate between 0.0035 and 0.0156 mg is fatal to the Japanese beetle.

The death rate of the beetles obtained from different sources in the field was practically the same as that of beetles reared in the insectary.

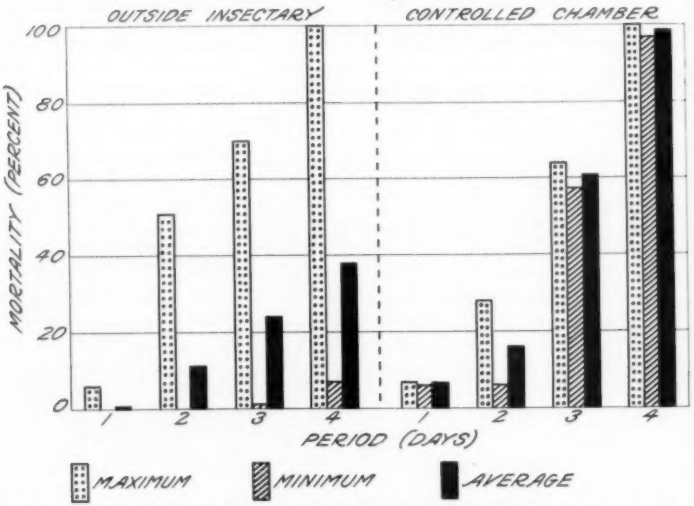

FIGURE 2.—Mortality of Japanese beetles when confined with sprayed foliage in wire cages in an outside insectary and in a chamber under controlled conditions.

It would appear, then, that beetles collected at random in the field from unsprayed plants should be as satisfactory for insecticide experiments as beetles reared in the insectary.

COMPARATIVE MORTALITY OF BEETLES IN CAGES WITH SPRAYED FOLIAGE AND WITHOUT FOOD

The results of the experiment in which insecticides were tested in wire cages in an open insectary are given in table 2 and also, in part, in figure 2. It is seen that at any given period there was considerable difference between the maximum and the minimum mortality. It is, therefore, apparent that the average mortality has no particular significance. Furthermore, since the average death rate of the beetles confined without food in most cases exceeded that of the beetles confined with sprayed foliage, there is some doubt as to whether the beetles in cages with the arsenical died from starvation or from poisoning, particularly since only a small portion of the beetles were on the foliage at any time and the feeding was limited.

TABLE 2.—*Mortality of Japanese beetles when confined in wire cages in the insectary with foliage sprayed with lead arsenate, and when confined in cages without food*

Period of observation (days)	Mortality of beetles					
	Confined with sprayed foliage			Confined without food		
	Maximum	Minimum	Average [a]	Maximum	Minimum	Average [a]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	6	0.0	0.3	20	0.0	3.1
2	51	.0	10.8	50	.0	8.2
3	70	1.0	23.5	100	.0	28.1
4	100	7.2	38.6	100	.0	56.1
5	100	25.0	51.9	100	.0	73.9

[a] As the frequency distributions from which the figures in this column were computed exhibit no definite central tendency, these figures cannot be considered as true averages, but they are included in order to show the discrepancy between them and the valid averages contained in subsequent tables which give the results of experiments carried on under controlled conditions.

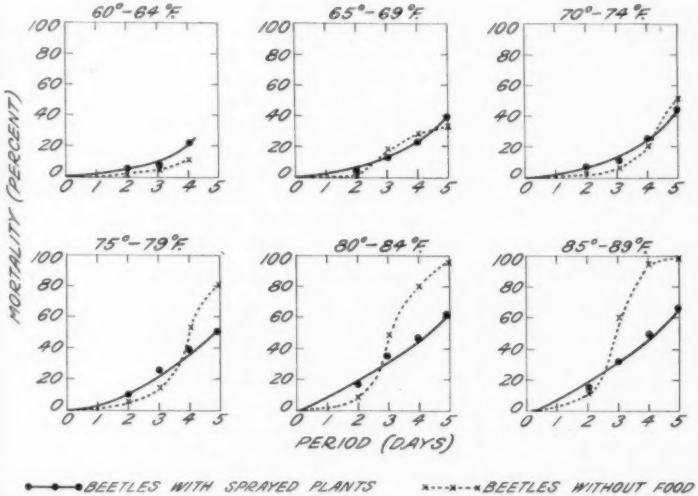

FIGURE 3.—Effect of temperature upon the mortality of Japanese beetles when confined in the open insectary in cages with sprayed plants and in cages without food.

EFFECT OF TEMPERATURE ON MORTALITY OF BEETLES

A general idea of the effect of temperature on the death rate in the open insectary was obtained in the following manner: The average temperature for the daylight hours of each day was determined in each of the 5-day test periods in each of the 51 experiments. The beetles surviving in cages with sprayed foliage, with unsprayed foliage, and without food were segregated into groups according to 5-degree ranges of these average temperatures, as follows: 60°–64°, 65°–69°, 70°–74°, 75°–79°, 80°–84°, 85°–89° F. The percentage mortality of the beetles with sprayed foliage and without food was determined for each day in the 5-day period at these temperature ranges according to the formula given on page 117. These data are

presented graphically in figure 3. There is a definite correlation between the average temperature of the daylight hours and the death rate. The final average mortality of beetles confined with sprayed foliage was 38.7 percent when the average temperature was 65°–69°, and 65.8 percent when the average temperature was 85°–89°. The average mortalities of the starved beetles under these conditions were 33.4 and 100 percent, respectively. At temperatures below 75° there was close agreement between the death rates of beetles confined with sprayed foliage and those kept without food; at higher temperatures the death rate of the starved beetles exceeded that of the beetles in contact with sprayed foliage after 3.75 days at 75°–79°, 2.75 days at 80°–84°, and 2.5 days at 85°–89°.

EFFECT OF RELATIVE HUMIDITY ON MORTALITY OF BEETLES

The increase in the death rate of the starved beetles above that of beetles with food at temperatures above 75° F. was probably due partly to the low relative humidity at midday which generally accompanies the higher temperatures in this locality. It would be expected that under these conditions, when the insects had no access to water or to a succulent plant, the evaporation on the surface of the bodies might reduce the moisture content to a point where survival was impossible. An attempt to correlate the relative humidity with the death rate was unsuccessful because of the rapid fluctuations in the relative humidity throughout the day. When the beetle had access to a succulent plant, a change in the relative humidity made little difference in the death rate, indicating that there was sufficient moisture in the foliage to maintain the moisture content of the insect's body practically independent of the surrounding atmosphere.

LIMITATIONS OF THE WIRE CAGE IN AN OPEN INSECTARY

The simple wire cage in an insectary, with no control of the atmosphere, is not a suitable environment for obtaining definite information on the effectiveness of different materials as stomach poisons against the Japanese beetle. The principal limitations are as follows: (1) The beetles pass a large part of the experimental period on the wire of the cages in attempting to escape; (2) the beetles do not begin to feed on the foliage until from 20 to 24 hours after introduction into the cages; (3) the fluctuations in temperature, relative humidity, and light modify appreciably the activity and death rate of the beetles, making it difficult to obtain comparable results in successive tests with any material; and (4) the high death rate of the beetles confined without food gives a reasonable basis for questioning whether the mortality in the cages with sprayed foliage should be attributed to poisoning or to starvation, particularly where the feeding of a number of beetles is limited to a few small punctures in the foliage. It seemed imperative to improve this method of testing stomach-poison insecticides before proceeding further with the experimentation.

DEVELOPMENT OF AN IMPROVED CAGE

Early in the spring of 1932 some preliminary experiments were conducted to observe the reactions in different types of cages of Japanese beetles that had emerged in the greenhouses. Some beetles were placed in bell jars with rose foliage. The temperature

was 80° F., and the relative humidity was maintained at approximately 70 percent by drawing air through saturated solutions of sodium chloride and then through the jars. A 75-watt bulb was suspended above them for illumination.[7] The beetles did not attempt to escape, as in the wire cages, but fed extensively on the foliage.

A bell jar is not well adapted to insecticide tests on an extensive scale, because of the necessity of aerating it artificially. Several types of cages were constructed in an attempt to retain the desirable features of the bell jars and to eliminate the necessity of drawing air continuously through them. It was found that wire on the sides or top of the cage was not desirable, as the beetles usually went to the wire and ignored the foliage. The beetle is negatively geotropic.

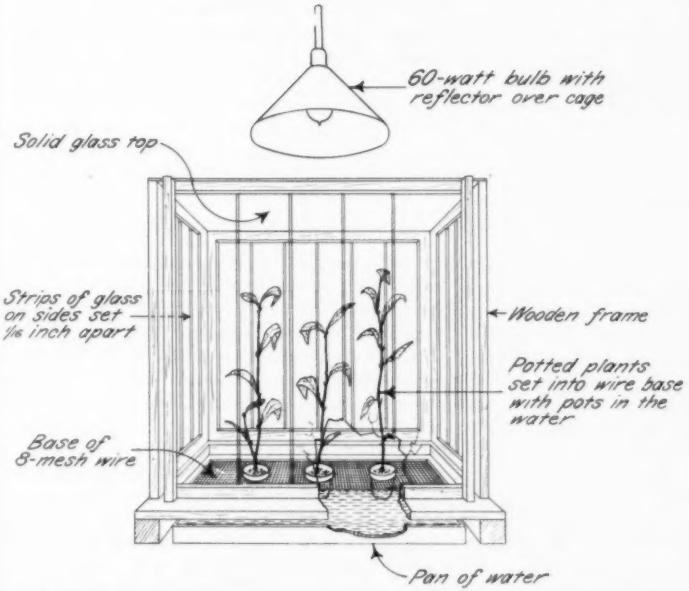
60-watt bulb with reflector over cage
Solid glass top
Strips of glass on sides set 1/16 inch apart
Wooden frame
Potted plants set into wire base with pots in the water
Base of 8-mesh wire
Pan of water

FIGURE 4.—Glass cage for testing stomach-poison insecticides against Japanese beetles.

It is usually found on the tops of trees, shrubs, and weeds, and when confined in a cage it tends to climb to the uppermost part of any wire or framework to which it can cling, and to remain on any part of the top or sides of the cage, encountered while flying, that can serve as a resting place.

From the experience obtained with the different types of cages, a glass cage was constructed which made it practically impossible for the insects to cling to the sides or top and induced them to congregate on the foliage of the plants. This glass cage is illustrated in figure 4. The top is covered with a sheet of glass; the sides are made of 1½-inch strips of glass spaced one sixteenth of an inch apart to provide ventilation. The cage is assembled in such a manner that the wooden frame does not project into the interior, except for a short distance at

[7] This experiment was carried on by F. W. Metzger, of the Japanese beetle labortory.

the top and bottom. The cage rests on a base covered with 8-mesh wire, through which circular holes are cut to receive potted plants. A pan of water is placed under the base. When the plants are placed in the cage, the lower parts of the pots are in the water, making it unnecessary to disturb the beetles to water the plants during an experiment.

DETERMINATION OF OPTIMUM CONDITIONS FOR TESTS

Experiments were conducted in the spring and summer of 1932 to determine the best temperature, relative humidity, and light for carrying on insecticide tests with the Japanese beetle.

OPTIMUM TEMPERATURE

It has been recognized for several years that the Japanese beetle is greatly affected by the temperature of the atmosphere, as it has no precise mechanism for regulating its body temperature. It is generally inactive below 70° F., is most active at 85° to 90°, and is quiescent at temperatures above 100°. From field observation and laboratory experimentation 90° appears to be the optimum for activity. At this temperature a cooling unit is rarely required in a constant-temperature chamber in this locality.

OPTIMUM RELATIVE HUMIDITY

Preliminary experiments were carried on at a temperature of 90° F. and at different relative humidities to determine the optimum humidity to be maintained in the cages. It was found that when the relative humidity was 0 to 5 percent the beetle was generally inactive and fed to a limited extent; at a relative humidity of 35 to 40 percent the beetle fed to a moderate degree but devoted most of its time to flying; and at a relative humidity of 90 to 95 percent it fed extensively and did little flying. These reactions under laboratory conditions are in general agreement with those observed in the field.

The period of survival without food is an important factor in insecticidal work with cages. One thousand beetles were confined without food in a bell jar in which the relative humidity at 90° F. was 0 to 5 percent, another group of an equal number was confined in a bell jar at 35 to 40 percent humidity, and a third group was confined at a humidity of 90 to 95 percent. The mortality of each group of beetles was determined at intervals. The results are presented graphically in figure 5. At a relative humidity of 0 to 5 percent, 50 percent of the beetles were dead at the end of 22 hours; at 35 to 40 percent relative humidity 50 percent were not dead until 48 hours had passed; and at 90 to 95 percent relative humidity 50 percent were not dead until the end of 81 hours. All the beetles were dead in 48 hours at the low humidity, some survived for 96 hours at a humidity of 35 to 40 percent, and some were alive after 144 hours at 90 to 95 percent relative humidity.

The influence of humidity has been ignored in the past in testing the effectiveness of stomach poisons against the Japanese beetle. It may not have so great an effect as temperature, but it does alter the results of successive tests with the same materials. From the results of these experiments it was concluded that a relative humidity between 90 and 95 percent is the optimum for conducting tests with stomach-poison insecticides against the Japanese beetle.

OPTIMUM ILLUMINATION

In the dark the Japanese beetle is practically inactive, although if it is on foliage it may feed a little; it is aroused to activity by illumination. Moore and Coles (7) observed that the beetle shows a negative geotropic response only when illuminated. It is well known that the Japanese beetle is affected by the length of the day and the intensity of the illumination. Light is essential to extensive feeding by this insect. Under controlled conditions artificial illumination is necessary. The kind of light to be used in climatic simulation should be determined by the response of the insect. It was planned to study the feeding response of the beetle to artificial yellow light from electric bulbs of different intensities and, if this source of light proved unsatisfactory, to experiment with light from other sources.

The response of the beetle to artificial yellow light of different intensities was studied in the special glass cages in a dark chamber.

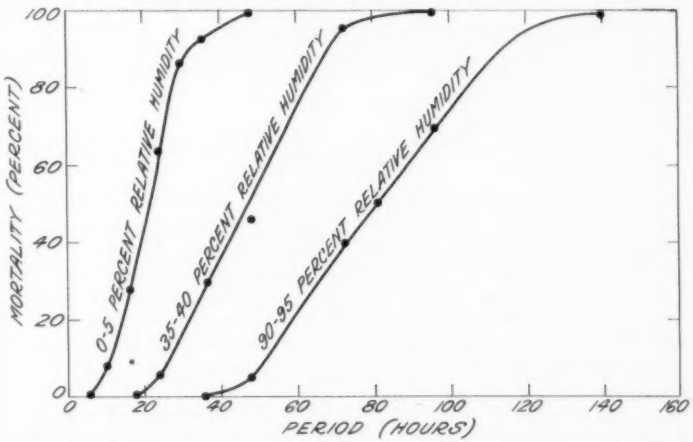

FIGURE 5.—Effect of relative humidity on the mortality of Japanese beetles kept without food at 90° F.

When the average intensity was less than 85 candle-meters, the insect was inclined to be sluggish; with an illumination of 85 candle-meters it moved readily and fed extensively on the smartweed in the cage; but the response was not greatly increased when the intensity was increased to 500 candle-meters. Moore and Cole (7) found that the beetle responded in 15.5 seconds to light of an intensity of 85 candle-meters, and by increasing the intensity to 3,276 candle-meters they reduced the time to 10.22 seconds. The activity of the beetles in the special glass cages illuminated with artificial yellow light of an intensity of 85 candle-meters was practically the same as in the field; the beetles collected in groups on the plants, fed extensively, mated freely, and deposited eggs in the soil about the roots of the plants.

This response of the Japanese beetle to artificial illumination is similar to that of grasshoppers, as shown by Parker (*10*), who found that the feeding response of the grasshoppers *Melanoplus mexicanus mexicanus* Sauss. and *Camnula pellucida* Scudd. in controlled cabinets

illuminated artificially with a 75-watt bulb was practically the same as the response in the field.

Inasmuch as a satisfactory feeding response was obtained with artificial yellow light of an intensity of 85 candle-meters, it did not appear necessary to experiment with illumination of greater intensity or to try other kinds of light.

An average intensity of 85 candle-meters was obtained by placing a 60-watt bulb in a flood-light reflector 6 inches above the cage. The intensity of the illumination in the cage varied with the distance from the bulb. On the bottom of the cage, 26 inches from the bulb, it was 40 candle-meters; it was 50 candle-meters 4 inches above the bottom, 60 at a height of 8 inches, 80 at a height of 12 inches, and more than 100 at a height of 16 inches. The distribution of light in this manner stimulated the insect to move to the foliage in the upper and lighter part of the cage.

These experiments indicated that the best environment in which to conduct controlled tests of stomach-poison insecticides on the Japanese beetle was one with a temperature of 85° to 90° F., a relative humidity of 90 to 95 percent, and continuous illumination from the top with artificial yellow light having an average intensity in the cage of 85 candle-meters.

CHAMBER WITH CONTROLLED TEMPERATURE, HUMIDITY, AND LIGHT

A satisfactory controlled chamber [8] was constructed by lining the walls and ceiling of a cellar with insulating wall board and installing heating and humidifying devices. The temperature is controlled by a commercial thermostat equipped with a rocking mercury valve. The sensitive element of the thermostat is so arranged that as the temperature falls the mercury flows to one end of a glass tube and closes the circuit between two electrical points; and when the temperature reaches 90° F. the mercury flows away from these electrical points, leaving the circuit open. The thermostat activates a centrifugal pump which forces water at 160° through a heating unit and starts a fan which draws air over the heating unit and returns it to the chamber.

The humidity is controlled by means of a silk-thread hygrostat, which by expanding and contracting activates a rocking mercury valve, operating the humidifier. The humidity is maintained at 90 to 95 percent by drawing the air through a fine spray of water and returning it to the chamber.

Each cage is lighted by a 60-watt bulb in a flood-light reflector placed 6 inches above the cage. The walls, ceiling, and benches are painted black to avoid reflection and to have the zone of most intense illumination in the cages.

COMPARISON OF RESULTS WITH LEAD ARSENATE IN THE INSECTARY AND IN THE CONTROLLED CHAMBER

A series of experiments were conducted to compare the results obtained with lead arsenate under these controlled conditions with those obtained in the insectary where there was no control of the atmospheric conditions.

[8] A. R. Whitcraft, laboratory mechanic, supervised the construction of this chamber and installed the equipment.

In the experiments in the controlled chamber the cages were maintained at a temperature of 90° F. and 90 percent relative humidity and were illuminated constantly throughout the period of observation. Beetles were collected from unsprayed foliage in the field. Fifty were placed in each of three cages with smartweed that had been sprayed with lead arsenate (4 pounds to 50 gallons of water), the same number were placed in three cages with unsprayed smartweed, and the beetles in a third group were confined in three cages without food. Three experiments were conducted during the season, with a total of 1,350 beetles. The number of beetles surviving in each cage was recorded every day for 4 days. At the end of the season the maximum, minimum, and average mortalities of the nine groups of 50 beetles confined with sprayed foliage and also of those confined without food were determined for each 24-hour period of observation according to the previously stated formula. These data are given in table 3 and figure 2.

TABLE 3.—*Mortality of Japanese beetles when confined in glass cages in the controlled chamber with foliage sprayed with lead arsenate and when confined in cages without food*

Period of observation (days)	Mortality of beetles					
	Confined with sprayed foliage			Confined without food		
	Maximum	Minimum	Average	Maximum	Minimum	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	6.5	6.0	6.2	2.0	0	1.0
2	27.6	6.1	16.6	0	0	0
3	64.5	57.0	61.0	28.6	17.8	23.0
4	100.0	97.4	99.0	60.5	38.6	48.8

The results obtained under controlled conditions were compared with those obtained under uncontrolled conditions in the insectary (table 2 and figure 2). It was found that the difference between the maximum and the minimum mortality at a given period was decreased considerably by conducting the experiments under controlled conditions. Whereas the difference between the maximum and the minimum mortality of beetles confined with sprayed plants in the insectary was 6 percent on the first day, 51 percent on the second day, 69 percent on the third day, 92.8 percent on the fourth day, and 75 percent on the fifth day, these differences were reduced to 0.5, 21.5, 6.5, and 2.6 percent, respectively, under controlled conditions. The maximum mortalities for the different periods under insectary conditions, when the temperature was high, compared favorably with those obtained in the controlled chamber. It was therefore believed that, if the experimental conditions in the insectary could have been maintained close to those at which these maximum mortalities were obtained, the results would have been in close agreement with those obtained in the controlled chamber.

When beetles were confined without food, the difference between the maximum and the minimum mortality was greatly reduced under controlled conditions (tables 2 and 3). In the insectary the difference between the maximum and minimum mortality was 20 percent on the

first day, 50 percent on the second day, and 100 percent on and after the third day. In the controlled chamber these differences were reduced to 2, 0, 10.8, and 21.9 percent, respectively. The death rate of the beetles confined without food in the controlled chamber, while not so high as that under insectary conditions, is, however, higher than is desired in a test of this type.

In view of the fact that the beetles consumed about 20 percent of the foliage sprayed with lead arsenate under controlled conditions, the mortality due to starvation probably had little influence on the results. It is possible, however, to spray the foliage with a material that will repel the beetles and prevent them from feeding. In such a case the mortality due to starvation becomes an important factor. It was considered desirable, before testing materials as stomach-poison insecticides by this method, to expedite, if possible, the feeding of

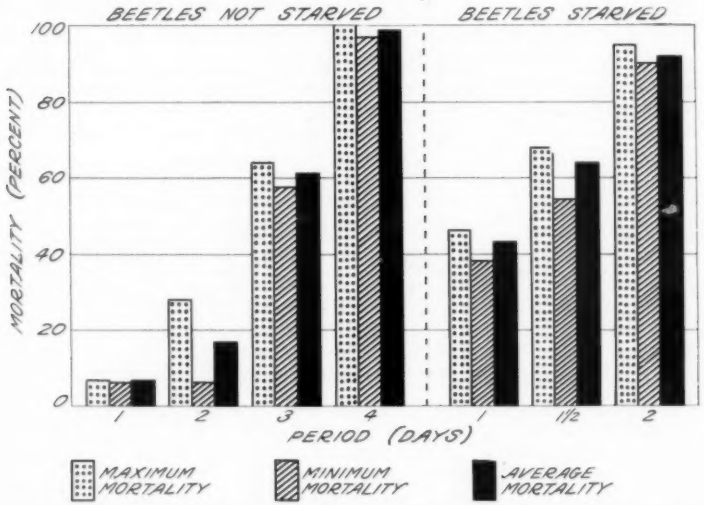

FIGURE 6.—Mortality of Japanese beetles placed in a controlled chamber with sprayed foliage immediately after collecting and after being starved for 3 to 6 hours.

the beetles on sprayed foliage and thus increase the death rate to reduce the factor of starvation to a minimum.

EXPEDITING INSECTICIDAL TESTS IN THE CONTROLLED CHAMBER

Beetles collected in the field and placed directly in cages did not gather on the foliage in maximum numbers until 20 to 24 hours after the test was begun. It was obvious that, if beetles could be induced to feed on the foliage in large numbers in a shorter time, the death rate of those confined with sprayed foliage might be expedited. Starving beetles for 3 to 6 hours before placing them in the test cages so stimulated their search for food that 75 to 80 percent gathered on the foliage within 9 hours.

Starved and unstarved beetles were placed in cages with plants sprayed with lead arsenate (4 pounds to 50 gallons of water), in cages

with unsprayed plants, and in cages without food to determine the effect of withholding food from the beetles for this period. The number of beetles used was 3,750, from 50 to 400 being used in each test. The percentage mortality of the beetles was determined in the usual manner. The results are given in table 4 and presented graphically in figure 6. With sprayed foliage practically complete mortality of the starved beetles was obtained in 2 days, whereas 4 days was required with beetles directly from the field. Starving beetles for a short time before using them in insecticide tests makes it possible to complete an experiment in half the time required with unstarved beetles. When confined without food for 2 days, the death rate of both the starved beetles and those directly from the field was so low as to be of negligible importance in insecticide tests.

TABLE 4.—*Effect of starving Japanese beetles on their mortality when confined in cages with sprayed foliage and when confined without food*

Time (days)	Mortality of beetles directly from field						Mortality of beetles starved 3 to 6 hours					
	Confined with sprayed foliage			Confined without food			Confined with sprayed foliage			Confined without food		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	6.5	6.0	6.2	2.0	0	1.0	46.7	37.7	43.0	-------	-------	6.4
1½	-------	-------	-------	-------	-------	-------	68.0	53.8	63.3	-------	-------	4.2
2	27.6	6.1	16.6	0	0	0	94.7	89.8	91.7	-------	-------	5.0
3	64.5	57.0	61.0	28.6	17.8	23.0	-------	-------	-------	-------	-------	-------
4	100.0	97.4	99.0	60.5	38.6	48.8	-------	-------	-------	-------	-------	-------

NUMBER OF BEETLES REQUIRED FOR A SATISFACTORY TEST

The number of insects used in a test with a stomach-poison insecticide is often taken as an indication of the reliability of the data. It is desirable to use as many individuals as possible in testing a material without making the method cumbersome. In order to determine the consistency of the data obtained with different numbers of beetles, groups of 50, 200, 400, and 500 that had been starved for 3 hours were placed in cages with plants that had been sprayed with lead arsenate. The maximum, minimum, and average mortality of these groups was determined at the end of 48 hours.

The results (table 5) indicate that the number of individuals makes little difference in the average mortality, provided that, when a small number are used, the experiment is repeated several times. The difference between the maximum and the minimum mortality did not change appreciably until 500 beetles were used as a unit. A group of 200 is a convenient unit for tests with the Japanese beetle, and this number is recommended as a standard unit for stomach-poison tests with this insect.

TABLE 5.—*Effect of the number of beetles in a cage on the consistency of mortality tests*

Beetles per cage (number)	Beetles in experiment	Mortality after 48 hours		
		Maximum	Minimum	Average
	Number	*Percent*	*Percent*	*Percent*
50	2,200	94.4	78.4	87.9
200	2,400	95.7	77.3	86.7
400	4,800	94.7	74.1	87.6
500	2,500	89.0	82.9	86.4

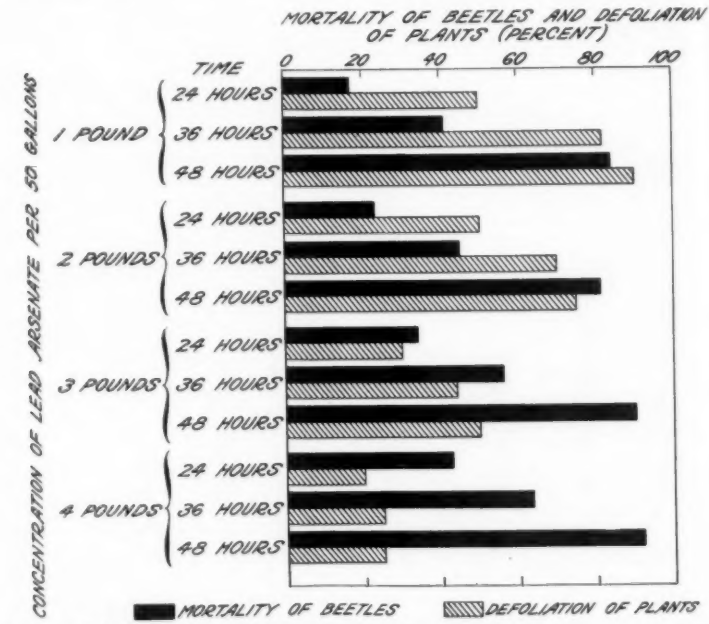

FIGURE 7.—Relation between the concentration of lead arsenate in the spray to the extent of feeding and mortality of Japanese beetles.

RELATION OF CONCENTRATION OF LEAD ARSENATE IN THE SPRAY TO FEEDING AND MORTALITY

A lead arsenate paste was prepared by triturating 50 parts of the powder with 2 parts of oleic acid and adding sufficient water to make the lead arsenate 50 percent by weight of the final product. The addition of the oleic acid gave a more uniform coating without injuring the foliage.

Smartweed plants in cages were sprayed with this paste at concentrations ranging from 1 to 4 pounds of lead arsenate to 50 gallons of water. Two hundred beetles, which were starved as previously described, were placed in each cage with the sprayed plants, and their mortality was determined after 24, 36, and 48 hours. The extent of feeding, as indicated by the degree of defoliation, was also estimated at these intervals. The results of these experiments are given in table 6 and presented graphically in figure 7.

TABLE 6.—*Effect of concentration of lead arsenate sprayed on foliage on the mortality and the extent of feeding of Japanese beetles*

Concentration of lead arsenate (pounds per 500 gallons)	Beetles in experiment [a]	Mortality of beetles									Degree of defoliation		
		After 24 hours			After 36 hours			After 48 hours			After 24 hours	After 36 hours	After 48 hours
		Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average			
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	3,200	20.6	13.1	17.1	51.9	33.3	40.9	86.4	82.1	84.1	50	82	90
2	3,200	26.7	18.1	22.9	46.8	38.6	44.8	84.4	74.0	81.0	50	70	75
3	2,800	36.6	28.6	33.6	59.1	53.0	56.3	93.0	87.7	90.1	30	44	50
4	2,800	46.5	37.7	43.0	68.0	53.0	63.3	94.7	89.8	91.7	20	25	25

[a] 200 beetles were placed in each cage.

The average mortality increased with the increment in the concentration of lead arsenate. At the end of 24 hours there was 25.9 percent difference in mortality between the high and the low concentrations, but after 48 hours the difference was only 7.6 percent. The indications are that if the experiment had been continued for a longer period there would have been no difference.

We may conclude from these data that the Japanese beetle continues to feed on foliage sprayed with lead arsenate until it feels the effect of the arsenic, and that the final mortality is practically independent of the quantity of arsenical residue on the foliage. In order to protect the plants, however, it is desirable that this residue be sufficient to affect the beetle before it consumes much of the foliage.

SUMMARY AND CONCLUSIONS

Testing the effectiveness of stomach-poison insecticides against the Japanese beetle in wire cages, without control of environmental conditions, is not a satisfactory procedure, because of the variation in the temperature, relative humidity, and light, and because the beetle spends a large part of the time on the wire of the cage attempting to escape. A special glass cage has therefore been developed in which there are no resting places at the top or sides and the beetle is attracted to the plants.

Comparable results have been obtained in successive tests with the same materials at a temperature of 85° to 90° F. and a relative humidity of 90 to 95 percent, under artificial yellow light of an intensity of 85 candle-meters.

A group of 200 individuals is a convenient unit for conducting stomach-poison insecticide tests with the Japanese beetle.

The results obtained with commercial acid lead arsenate by this procedure confirm the conclusion that this compound is an effective stomach-poison insecticide against the Japanese beetle. The concentration of the arsenical governs the extent of feeding on the foliage and for a certain period the mortality of the beetles, but at the end of this period mortality is independent of the lead arsenate concentration.

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FROG-EYE (CERCOSPORA DIAZU MIURA) ON STEMS, PODS, AND SEEDS OF SOYBEAN, AND THE RELATION OF THESE INFECTIONS TO RECURRENCE OF THE DISEASE[1]

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INTRODUCTION

The leaf-spotting fungus, *Cercospora diazu* Miura, frequently attacks certain varieties of soybean, *Soja max* (L.) Piper, grown in the Southern States. The name "frog-eye" [2] has been given to the disease produced by this fungus on the soybean. The first authenticated observations of frog-eye on soybeans in the United States were made in 1925, when the disease was found in five Southern States. Announcement of the discovery of the disease in North Carolina and of the probable identity of the causal fungus with *Cercospora diazu* Miura, previously described as the cause of a disease of soybean in Manchuria and Japan, was first made by Wolf and Lehman [3] in 1926. A detailed description of the disease and of *C. diazu* with an account of the isolation and inoculation tests proving the causal relation of the fungus to the disease was published by Lehman [4] in 1928. At that time very few lesions had been found on stems and none at all on pods. Since then many infections of the frog-eye disease have been observed on pods and stems in a number of plantings. In the present paper the writer recounts briefly the symptoms on stems, describes the disease as it appears on infected pods, notes the extent and character of seed infection, gives certain data on longevity of the causal fungus in culture, presents the results of tests showing that the disease may overwinter in leaves and stems left in the field following a diseased crop, and shows that the disease may be initiated in new territory by use of infected seed.

APPEARANCE OF THE DISEASE ON STEMS

Stem infections are less numerous and somewhat less conspicuous than those on leaves. Typical stem lesions have been observed on seedlings growing in large test tubes, but none have been seen on seedlings growing in the field. They appear in the field in large numbers only in late fall when a considerable quantity of inoculum has accumulated on the leaves and the plants are maturing seed. At this time the stems are ripening and are possibly less resistant to fungal invasion than younger stems. Also the lower air temperature and greater abundance of moisture on the plant surfaces at this time of year may constitute conditions more favorable for infection than the hotter summer days earlier in the season.

Stem lesions (fig. 1, *A*) are usually 2 to 4 times as long as broad, the greater dimension lying in the direction of the long axis of the stem.

[1] Received for publication Aug. 3, 1933; issued March 1934.

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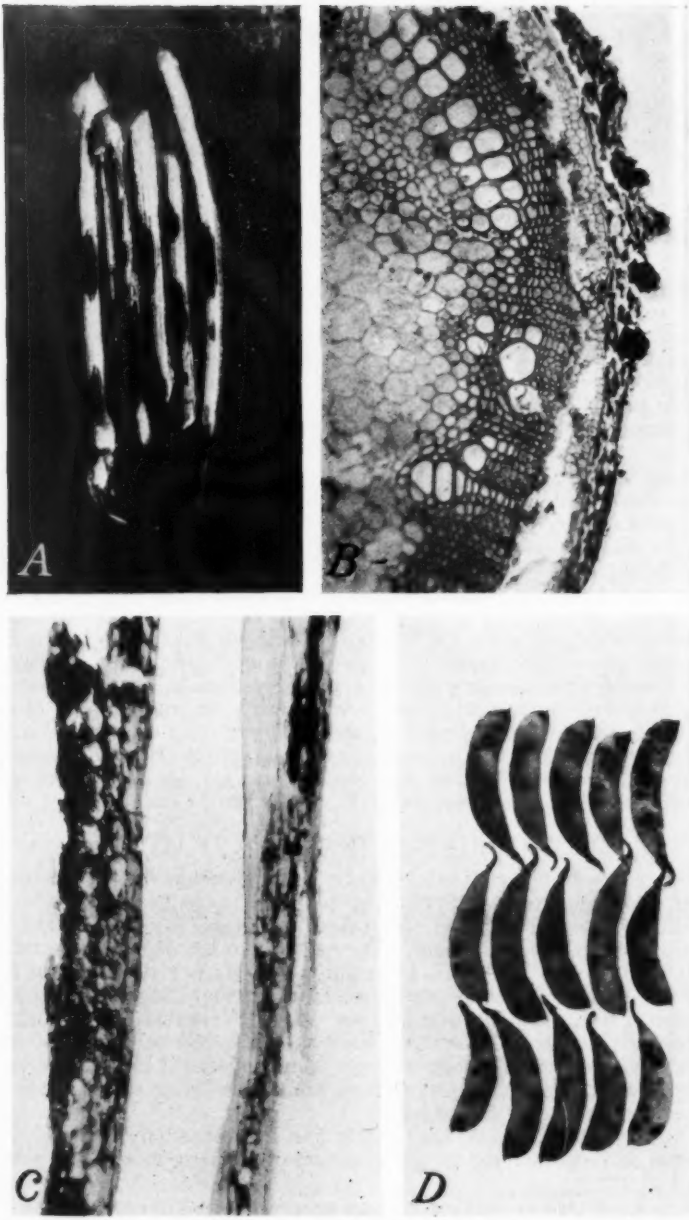

FIGURE 1.—*A*, Stem lesions (yellow filter used in photographing) approximately natural size. The stem of greatest thickness had an actual length of 65 mm. Its color was a ripe brown. The lesion on this stem was 15 mm long and had an outer marginal band of reddish brown, darker than the ripe brown of undiseased tissue. Within this was a narrow band of light brown very little different from undiseased tissue in color. The large central area had been darkened by formation of numerous minute stromata. *B*, Microtome section of stem cut through a frog-eye lesion. Note the darkly stained mycelium in the cortex and three minute stromata protruding through the epidermis. × 100. *C*, An unstained longitudinal section of a stem lesion. Note the pronounced development of mycelium in the cortex and in the phloem beyond the intervening band of sclerenchyma. This section was cut between the lines of a tangent and a radius, thus the sclerenchyma appear excessively thick. *D*, Lesions produced by infection of *Cercospora diazu* on soybean pods. Collected November 1, 1928.

They frequently spread one fourth to one half the distance around the infected stem, but seldom does a single lesion girdle the stem. The central portion of the diseased area may be flattened or slightly sunken. On very young lesions the whole diseased area is some shade of red, or the central portion may be red bordered by a narrow zone of dark brown to black beyond which is the normal green of the undiseased tissue. As the lesion enlarges, the central area loses its red color, becoming brown, then pale smoke gray. There may or may not remain a narrow intervening band of red between the gray central area and the dark-brown outer zone. Minute black stromata, sometimes bearing conidiophores with conidia, are often visible in large numbers in the gray centers of old lesions. Sometimes dark-colored mycelium develops so abundantly as to cause the entire surface of older lesions to become black.

Sections cut through diseased areas on stems show an abundance of mycelium in the cortical tissue (fig. 1, *B*, *C*). Mycelium is prevalent in the cortical region including the endodermis. It tends to develop in strands of two to several hyphae, which grow more often in the intercellular spaces than through the cells. Hyphae aggregate in the form of minute stromata at numerous places within or immediately beneath the epidermis. These rupture the overlying cell membrane and extrude as black bodies of pin-point dimensions (fig. 1, *B*). A more or less continuous flattened stromatic development is shown in figure 1, *C*. Just within the endodermis is a layer of sclerenchyma fibers constituting the outer portion of the pericycle. Only rarely are hyphae found in the lumina of sclerenchyma cells or between them. Sometimes the fungus passes through the sclerenchyma and permeates the thin-walled parenchyma of the pericycle and the adjacent phloem and cambium. This passage is made by way of occasional thin-walled cells which separate the several flattened bundles of thick-walled cells constituting the all-but-continuous band of sclerenchyma under the cortex. More often than otherwise the fungus fails to cross the sclerenchyma. Even then, however, the cells of the phloem and cambium lying beneath the invaded cortex are usually found moribund or completely disorganized. Presumably, harmful substances excreted by the fungus growing in the cortex or by-products of the reaction between the fungus and cortical cells diffuse through the sclerenchyma and kill tissues beyond reach of the fungus itself. Likewise, in the cortex the normal condition and the staining reaction of the host cells at the margin of a given lesion are changed for a considerable distance beyond the area actually invaded by hyphae.

LESIONS ON PODS

Lesions have been seen in large numbers on pods of only one variety, the Otootan. This variety does not begin setting pods until late in the growing season, hence pod infections do not become evident until late fall. An earlier fruiting variety, the Laredo, is approximately equal to Otootan in susceptibility of foliage, but comparatively few pod lesions have been found on heavily diseased Laredo plants. This variety matures 30 days earlier than Otootan and at a time when air temperatures are high and relatively little dew accumulates on plants. It is thought that the differences in temperature and humidity, particularly the length of time atmospheric moisture persists on the plants as dew at the time the pods are developing and maturing, may

account for this observed difference in the amount of disease found on pods of these two varieties. This belief is supported by the observation that on Otootan the first-formed pods are relatively free of disease as compared with pods formed a week or 10 days later, although many sporulating lesions have long been present on the foliage of the same plants. Usually pod lesions do not become much in evidence until pathological defoliation is well under way. There is also a marked increase in number of cauline lesions at this time.

Lesions on pods are usually quite circular (fig. 1, *D*). However, those which develop in contact with the dorsal or ventral suture tend to enlarge more rapidly along the suture, and those which arise from two or more points of infection and coalesce are irregular in outline. Single lesions vary in diameter from 1 to 4 mm, and as many as 14 have been counted on one side of a single pod. Young lesions on green pods are brown, often with a tint of red. A uniform brown color may persist over the lesion as the spot enlarges, but usually lighter colors develop in the center of the spot, so that on dry, ripe pods the diseased area is seen as a light-brown or gray spot encircled by a narrow dark-brown ring. Thus the so-called "frog-eye" aspect is characteristic of the disease on pods, and to some extent on stems, as well as on leaves.

The surfaces of lesions on pods are usually somewhat sunken because of the collapse of the diseased cells beneath. Figure 2, *A*, a section at the margin of such a lesion, shows the degree of shrinkage in the diseased area. Hyphae advancing within the tissues near the margin of a pod lesion are shown in figure 2, *B*. Nearer the central portion of the lesion, hyphae are much more abundant and tend to form dense stromalike aggregates similar to, but usually smaller than, those found on stems.

When pods bearing lesions of the frog-eye disease are opened, the discoloration marking the location of the infected area may be seen on the inner pod-wall tissues. The pod wall of the soybean is lined with a very thin membrane of hyaline cells. This membrane, which more or less completely invests the seed in each compartment of the pod and lies between it and the pod wall, usually comes free of the seed, leaving the latter smooth and shining when shelled from ripe healthy pods. In diseased pods the portion of this membrance which lies between a lesion and the seed coat often does not shed off from the seed, but clings to the seed coat, marking the point where the seed came in contact with the diseased portion of the pod wall (fig. 2, *D*). This serves as a means of identifying seeds from diseased pods. It is not an infallible sign of seed infection, however, since portions of membranes sometimes adhere to seed coats from other causes than parasitic invasion. Mycelium may usually be found by a microscopic examination of the portion of this membrane lying between the seed coat and the diseased tissue of the pod wall.

Macroscopic examination of seeds from diseased pods reveals no definite lesion or marked discoloration. However, there is often evident a slight depression in the portion of a seed which developed in contact with a pod-wall lesion, and usually the surface of the seed at this place is noticeably lacking in that smoothness and luster characteristic of healthy seed coats. This seed-coat feature, together with adhesion of small portions of the white investing membrane referred to above, appears to be fairly characteristic of seeds that develop in

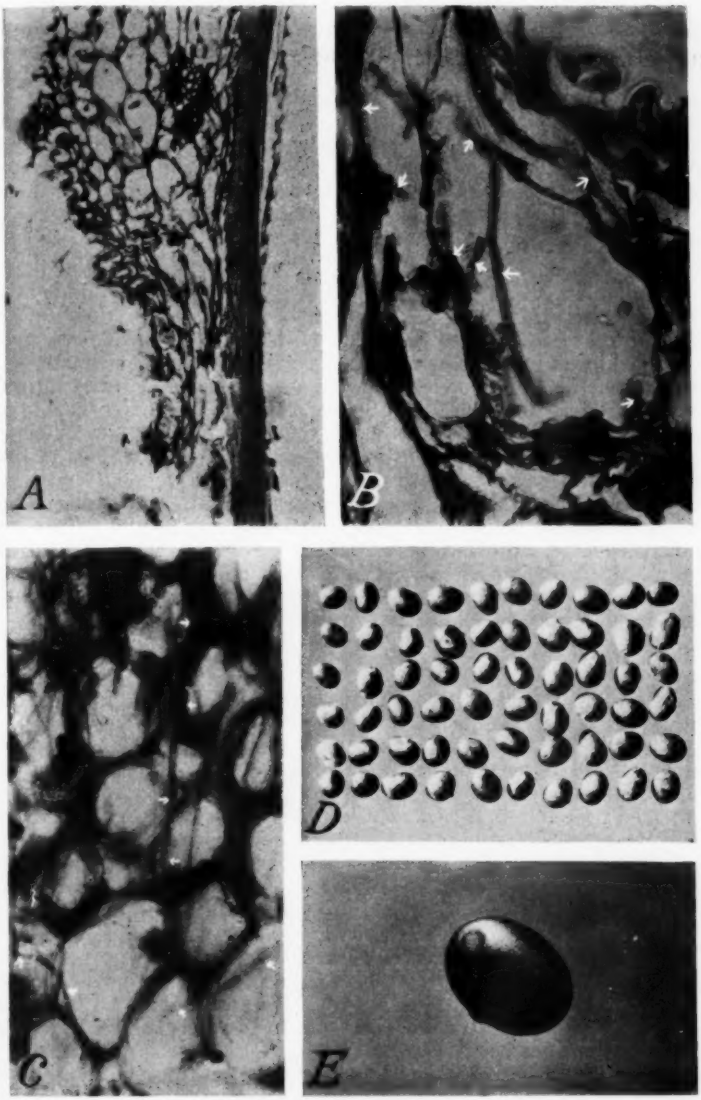

FIGURE 2.—*A*, Section of pod wall at margin of a frog-eye lesion showing sunken character of lesion due to shrinkage of diseased tissue. *B*, Mycelium in diseased tissues near the margin of a pod-wall lesion. × 600. *C*, Hyphae of the frog-eye fungus in the thin white membrane which lines the pod wall. In threshing, this membrane parts from seeds which have ripened normally, leaving them smooth and shiny, but portions of it frequently adhere to seed coats at the places where they developed in contact with pod-wall lesions. × 500. *D*, A group of soybean seeds taken from pods bearing lesions of the frog-eye disease. Portions of the pod-wall lining are adhering pathologically to the coat of each seed. This is obscured by high lights on some of the seeds but is plainly evident on others. × ⅔. *E*, A soybean showing the depression which often marks the place where the seed lay in contact with a pod-wall lesion. None of the diseased pod-wall lining has stuck to this seed. Actual length of seed 6 mm.

contact with pod-wall lesions and may be of some value as a criterion in estimating the amount of infection in different lots of seed. Figure 2, *D* shows a group of seeds that developed in direct contact with diseased areas of the pod wall. The place of contact is indicated either by a depression in the seed coat, or by the adhesion of portions of the thin white membrane, or by both. Figure 2, *E*, shows a single seed enlarged four and one half diameters to give a better view of the seed-coat depression. Macroscopic examination of the portions of the embryo lying in contact with the depressed portions of the seed coat gives no indication that the frog-eye fungus has penetrated embryonic tissues.

ISOLATIONS

Conidiophores and conidia are often seen on pod-wall lesions. In size and shape these are like those of *Cercospora diazu* that develop on leaf and stem lesions. A number of isolations have been made from both stem and pod-wall lesions. The usual procedure has been to wash the diseased portion in tap water, dip momentarily into 95-percent alcohol, immerse 40 to 60 seconds in 1:1,000 mercuric chloride solution, then plant the tissue on plain acidified agar. Nearly all the lesions tested in this way yielded a *Cercospora*, many times in pure culture, but often mixed with other fungi, chiefly *Penicillium*, *Alternaria*, and *Colletotrichum*. The *Cercospora* isolated from stem and pod lesions proved to be morphologically like the strains of *C. dizau* previously isolated from lesions on leaves.[5] The pathogenicity of the strains of *Cercospora* isolated from stems and pods was tested by inoculations on soybean leaves and pods in the greenhouse. These tests are described in a later paragraph.

SEED INFECTION

As previously noted, pod-wall lesions often lie in direct contact with seeds within the diseased pods. No open lesions are visible on the seed itself, there being at most only a slight depression or roughening of the seed coat at the place of contact with the diseased pod wall. Notwithstanding this lack of any conspicuous sign of infection, it seemed probable that seed infection might have occurred. Accordingly, tests were made to determine if the fungus had entered the seeds. In these tests, pods bearing well-developed lesions were selected and only those seeds that lay in contact with pod-wall lesions were used. The pods were washed under the tap, immersed in alcohol for a few moments, removed to mercuric chloride solution, 1:1,000, for 1 to 2 minutes, and finally rinsed in sterile water. The pods were then opened, and the seeds removed with sterile implements and placed on agar media. In certain tests a supplementary disinfection was given the seeds after removal from the pods. Table 1 gives the results of these tests.

The results of tests 1, 2, and 3 show that the frog-eye fungus had passed through the pod wall and had made intimate contact with a high proportion of the seeds selected. Test 3, in which seed coats and embryo were tested separately, indicates that the fungus may have been present on, or in the embryo of, at least one seed. However, since in this test the seed itself was given no disinfective treatment, there remains the possibility that, in the operation of removing the

[5] WOLF, F. A., and LEHMAN, S. G. (See footnote 3.)

seed coat, mycelium was transferred mechanically from the seed coat to the embryo. The treatments with alcohol and mercuric chloride in tests 4 and 5 reduced the recoveries of the fungus to 1 seed in 11 and 1 in 16, respectively. These tests indicate that the frog-eye fungus lies on or near the surface of infected seeds but may occasionally penetrate to the embryo. Additional evidence of internal infection of seeds will be presented in connection with certain seed-treatment tests described in the following paragraphs.

TABLE 1.—*Proportion of seeds yielding Cercospora when taken from diseased pods*

Test no.	Treatment given seeds after removal from disinfected pods	Total seeds	Number of seeds infected	
			On seed coat	In embryo
1	None	7	4	(a)
2	Do	28	23	(a)
3	Do	9	9	1
4	95 percent alcohol 5 seconds, $HgCl_2$, 1:1,000, 15-30 seconds, sterile water, 3 changes.	11	1	0
5	50 percent alcohol 20 seconds, $HgCl_2$, 1:1,000, 40 seconds, sterile water, 4 changes.	16	1	0

a Seed coats not separated from embryos, planted with coats intact.

TABLE 2.—*Proportion of seeds yielding Cercospora after harvest from infested field and following certain treatments*

[Germinated in large test tubes on plain 1 percent agar containing 0.2 percent of sugar; seed of Ototan harvested October 1928]

Test no.	Lot no.	Classification	Treatment	Seeds in lot	Germinated	Seeds yielding *Cercospora*	Seeds contaminated with other fungi or bacteria	Seeds originally selected as diseased
				Number	*Percent*	*Percent*	*Percent*	*Percent*
6	1	Strongly diseased.	70-percent alcohol 6 minutes; washed through sterile water, 3 changes.	50	98	14	42	
	2	do	None	50	98	2	98	
7	1	Diseased	70-percent alcohol 1 minute; sterile water, 3 changes.	50	90	6	54	13.3
	2	do	$HgCl_2$, 1:1,000, 10 minutes; sterile water, 3 changes.	50	98	10	26	
	3	do	70-percent alcohol 1 minute; $HgCl_2$, 1:1,000, 10 minutes; sterile water, 3 changes.	50	98	6	6	
	4	do	40-percent formaldehyde, 1 part in 320 parts water, 60 minutes; sterile water, 3 changes.	50	86	0	78	
	5	do	None	50	92	0	100	
8	1	do	$HgCl_2$, 1:1,000, 60 minutes; sterile water, 3 changes	50	98	4	18	13.1
	2	do	70-percent alcohol 1 minute; $HgCl_2$, 1:1,000, 60 minutes; sterile water, 3 changes.	50	96	4	8	
	3	do	Semesan solution, 0.25 percent, 60 minutes; sterile water, 3 changes.	50	98	6	42	
	4	do	70-percent alcohol 1 minute; Semesan solution 0.25 percent, 60 minutes; sterile water, 3 changes.	50	98	2	8	
	5	Not diseased.	$HgCl_2$, 1:1,000, 10 minutes; sterile water, 3 changes.	50	100	0	10	

An attempt was made to determine if one can by inspection recognize the diseased seeds and determine the probable percentage of infestation in a given lot of threshed seed. For this purpose seed

known to have come from an infested field were used. The usual procedure was to take at random one or more handfuls of seed from the bag. This seed was then sorted into two lots, one containing all seed judged to be diseased, the other those judged to be disease-free. The criterion of infection or contamination was the presence of a dulled, slightly roughened, often slightly depressed area on the seed coat. To this area a fragment of the thin white pod lining might or might not be clinging more or less tenaciously. The seed were germinated in large cotton-plugged test tubes containing plain agar media with 0.2 percent of sugar. The results of these tests are given in table 2.

In test 6, 100 seeds which showed the most pronounced evidences of disease out of a total of 576 were selected. The frog-eye fungus grew from 14 percent of the seeds which had been given the alcohol treatment. The untreated seeds of lot 2 were to all appearances equally as badly diseased as those of lot 1, but the frog-eye fungus appeared on only 2 percent of them when put to germinate although they had been given no preliminary disinfection. This unexpected result was probably due to the suppression of the comparatively slow growing frog-eye fungus by the heavy cultures of contaminating fungi in the tubes of lot 2. A much higher percentage of the tubes was contaminated in lot 2 than in lot 1 as was to be expected since lot 2 had received no treatment with a disinfectant.

The random sample taken for test 7 contained 2,138 seeds. When this sample was sorted, 286 seeds, or 13.3 percent, were found to bear the seed-coat markings taken as evidence of their having come from diseased pods. These diseased seeds were then divided into 5 lots of 50 seeds each and given the treatments indicated in table 2. In this test, lots 1, 2, and 3 yielded 6, 10, and 6 percent of diseased seed, respectively. The frog-eye fungus was not recovered from the seeds, of lot 4 nor from lot 5. Here again failure of the frog-eye fungus to appear in the untreated lot 5 was probably due to its complete suppression in competition with other contaminating fungi and bacteria. In lot 4 both the inhibiting action of contaminating organisms and the toxic action of formaldehyde may have operated to prevent the appearance of the frog-eye fungus. Judged by the high percentage of tubes infested by contaminating organisms, the formaldehyde treatment was not an effective seed treatment, yet its action of formaldehyde may account for failure of the frog-eye fungus in the uncontaminated tubes.

In test 8 a random sample containing 2,080 seeds was taken. Of these, 13.1 percent, or 273 seeds, showed the seed-coat symptoms, indicating that they had come from diseased pods. Four lots of 50 seeds each were taken from the diseased seeds. As indicated in table 2, the diseased seed were given somewhat more severe treatments than in previous tests in order to determine if the fungus might be inactivated in all the diseased seeds. Lot 1 was soaked in 1:1,000 $HgCl_2$ solution for 60 minutes, lot 3 in 0.25 percent Semesan solution for 60 minutes, and lots 2 and 4 were given a 1-minute soaking in 70-percent alcohol preliminary to the 60-minute soaking in the mercuric chloride or Semesan solution. The frog-eye fungus grew from 4, 4, 6, and 2 percent of the seeds in lots 1, 2, 3, and 4, respectively. Lot 5 consisted of 50 seeds from the undiseased portion of this seed sample. This lot was also treated with mercuric chloride solution to check

development of contaminating fungi in the germination tubes. Frog-eye fungus was not obtained from any seed in this lot.

The results of the seed-treatment tests show that it is possible to determine by inspection whether or not any considerable proportion of the pods from which the seeds came were infected with frog-eye. However, the percentage of threshed seeds from which the frog-eye fungus has been recovered in these tests has been low. For example, in test 7 (table 2), 13.3 percent of a sample taken at random were judged by inspection to be diseased. In the germination test only 6 percent of the seeds in lots 1 and 3 and 10 percent in lot 2 produced cultures of the frog-eye fungus. This is equivalent to only 3.85 percent of the 286 seeds selected as obviously diseased. Evidently this low percentage of recovery of the frog-eye fungus is due to the use of the seed disinfectant before putting the seed to germinate and does not mean that the criteria used in identifying diseased seeds are untrustworthy. This explanation is supported by the results given in table 1. In tests 1, 2, and 3, the seeds themselves were given no disinfecting treatment but were taken from the interior of diseased pods which had been treated with disinfectants before opening. *Cercospora diazu* grew from an average of 81.8 percent of these seeds. On the other hand, this fungus grew from less than 10 percent of the seeds in tests 4 and 5, in which the seeds were taken from beneath pod-wall lesions but were treated before being put to germinate. The use of the disinfectant not only more or less effectively controls contaminating fungi but also kills the frog-eye fungus on a considerable proportion of the seeds.

It is desirable to make germination tests on agar media. The seed sprout well on moist cotton or other nonnutrient media, but here the fungus on many seeds does not find enough nutriment in the seed-coat tissues to permit development to a point where it can be identified. On the other hand, this fungus often grows where the infected seed has come in contact, even though only momentarily, with moist agar when it does not develop on the seed coat. When seeds can be taken directly from pods the agar tube method gives satisfactory results; but when threshed seeds are used, contaminating organisms so overgrow and suppress the slow-growing frog-eye fungus as to make satisfactory results with this method impossible. The use of nutrient agar as the medium of germination makes it necessary that the seeds be given a preliminary treatment with a surface disinfectant to suppress contaminating organisms, and this in turn undoubtedly destroys the frog-eye fungus on that portion of the seeds that are only superficially infected. The infection percentages obtained by this method of seed testing must be taken to represent only a small fraction of the seeds with which the frog-eye fungus is in intimate contact. The seed tests indicate that in a relatively large percentage of the seeds the infection is so superficial as to be killed by disinfectants. This infection is real, however, and may initiate disease when untreated seed are planted. Experience indicates that where as many as 10 percent of the seeds show visible marks of disease practically 100 percent of the leaves of the plants from which the seed came were infected, each with numerous lesions.

The tests recorded in table 2 constitute additional evidence of the nature of seed infection. Such severe treatments as 70-percent alcohol for 1 minute followed by soaking in 1:1,000 solution of mercuric

chloride for 10 and 60 minutes, and by 0.25 percent Semesan for 60 minutes failed to eradicate the disease. The fungus in a small percentage of the seeds is evidently located deep within the seed-coat tissues, between the seed coat and embryo, or actually in the tissues of the embryo. Tests 3, 4, and 5 indicate that penetration of embryo tissues occurs in only a very small percentage of cases, if at all.

INOCULATIONS

Upon the discovery of frog-eye disease on stems, pods, and seeds, and the subsequent isolation of *Cercospora diazu* therefrom, it seemed desirable to use these isolants in the production of the frog-eye disease on leaves, and, conversely, to use isolants from leaf lesions in the production of the disease on pods and seeds. Accordingly a number of inoculation trials were made. The first of these were made with cultures isolated from seeds and pod-wall lesions on the unifoliate leaves of Otootan seedlings. The inoculations were made by rubbing the fruiting surface of the fungus colonies growing in Petri dishes over the surface of the leaf. This was done gently so as to sow the leaf surface with conidia but leave little or no mycelium thereon. One unifoliate leaf of the pair on each plant was inoculated, the other of the pair being left as a check. The inoculated plants were kept in a humid atmosphere under bell jars for 3 days, after which time the bell jars were removed. All leaves, 4 in number, thus inoculated with isolants from pod-wall lesions, and all leaves, 8 in number, inoculated with isolants from seeds produced numerous lesions typical of those caused by the frog-eye disease on leaves in the field. By the end of 20 days from the date of inoculation some of the inoculated leaves were becoming chlorotic and were dying from the large number of lesions. The uninoculated check leaves, paired on each plant with inoculated leaves, were healthy, green, and larger than the corresponding inoculated leaves.

In another test, inoculation with cultures isolated from pod-wall lesions were made on several leaves of a large plant of the Biloxi while uninoculated leaves remained healthy. Successful reisolations variety. Typical frog-eye spots developed on the inoculated leaves, by approved methods were obtained from 7 out of 9 lesions taken from the inoculated leaves.

In a third test, a culture isolated from leaves and another isolated from stems were used in inoculating pods, stems, and leaves. A spore suspension was made by washing the surface of a tube culture with 5 cc of tap water. This was then applied to the parts inoculated by gentle rubbing with the fingers. Check plants were rubbed in the same manner, only tap water being used for this purpose. At the end of 19 days numerous typical lesions were present on leaves inoculated by spore suspensions from each of the cultures, while check plants growing in the same pots remained free of disease. No pod or stem lesions had developed by the twenty-third day, but slightly more than half the inoculated pods had died. Since this had also occurred on the check plants the dying of these parts was taken to be due not to disease but to injury in the operation of inoculating or to ineffectual pollination. When the plants were examined again 8 days later, 6 out of 17 of the remaining inoculated pods bore disease spots faintly or closely resembling those on infected pods in the field, while pods on check plants remained free of disease.

The diseased pods produced by the artificial inoculations described were used in reisolation tests. The pods were dipped for 10 to 15 seconds in 95-percent alcohol, then for 1 minute in 1:1,000 mercuric chloride, and finally rinsed in sterile water. The lesions were cut out and placed on potato agar. Also a number of the seeds which lay directly beneath pod lesions were removed with sterile implements and without further sterilization placed on nutrient agar in test tubes. *Cercospora diazu* was obtained from 9 of the 14 pod-wall lesions used. The plates in which the remaining 5 pod-wall lesions were planted became overgrown with *Rhizopus* sp. before the frog-eye fungus had time to develop in them. Three of eight seeds taken from these artificially inoculated pods yielded cultures of the frog-eye fungus. The mycelium of the fungus was quite insecurely attached to the seed coats, for colonies of the frog-eye fungus arose not only on the seed coats but also at several places where the infected seed touched the surface of the nutrient medium in rolling down the slope of the agar slant in the tubes used.

LONGEVITY OF THE FROG-EYE FUNGUS

In a previous paper [6] the author pointed out that conidia of the frog-eye fungus can survive long periods of storage in an air-dry condition. It was shown that a small proportion of the conidia were still viable when taken from leaves which had been kept dry in the laboratory for 94 days from the date of collection. Observations not previously reported show that the mycelium of the frog-eye fungus is also very long-lived in culture media. In making transfers of the fungus, old cultures have at various times been used. The usual procedure in making new cultures was to transfer a small quantity of mycelium and such spores as might be present either from the surface or edge of the old colony to fresh media. On one occasion transfers were made in this way from cultures which had been for 395 days on potato-glucose agar in tubes of 18 mm internal diameter. After the lapse of several days these transfers showed no evidence of growth. The entire quantity of substratum and mycelium of the old cultures was then removed and pressed into fresh potato-glucose agar in tubes. When transfers of the old cultures, now 405 days old, were made in this way, 9 of 10 cultures were found to be viable. At the time these transfers were made the old cultures were to all appearances thoroughly air-dry and occupied only a small fraction of their original volume. The cultures usually attain this air-dry condition in the course of 120 to 180 days. Table 3 shows the results of this and other tests of a similar nature. The frog-eye fungus has been found still viable in cultures as old as 493, 502, and 519 days. Three cultures which had reached an age of 919 days, and 15 which ranged from 979 to 1,731 days could not be revived by the method used here. The hot-agar method described by Povah [7] was used without success on five cultures ranging in age from 1,001 to 1,768 days. Apparently under the conditions of this test the frog-eye fungus will live on potato-glucose agar, and perhaps also on cooked rice, as long as, or longer than 519 days, but not so long as 919. It is judged that during approximately 400 of the 519 days the fungus remained alive, the substratum in which the fungus was embedded contained only such

[6] LEHMAN, S. G. See footnote 2.
[7] POVAH, A. NOTES UPON REVIVING OLD CULTURES. Mycologia 19: 317-319. 1927.

meager quantities of water as could be held against evaporation in the dry air of the laboratory. It seems probable that the fungus might live equally long in diseased leaf and stem tissues constituting the debris left from a diseased crop.

TABLE 3.—*Longevity of Cercospora diazu in culture*

Strain or isolation no.	Age at time of transfer	Cultures transferred	Cultures still viable	Strain or isolation no.	Age at time of transfer	Cultures transferred	Cultures still viable
	Days	*Number*	*Number*		*Days*	*Number*	*Number*
1 Re 1c	221	2	2	2–4	519	1	1
4C	341	1	1	10	919	1	0
1–3	405	4	4	Re 1	919	2	0
1–3 [a]	405	4	4	1	979 (and older)	15	0
2–2	405	2	1	7 [b]	1,001	2	0
1–4–5	493	2	2	4C–4 [b]	1,768	3	0
1–4	502	4	4				

[a] Growing on cooked rice; all other cultures grew on potato agar containing 2 percent of glucose or dextrose.
[b] Hot-agar method.

OVERWINTERING OR SEASONAL SURVIVAL ON INFECTED LEAVES AND STEMS

Observations made early in the writer's experience with frog-eye of soybean gave rise to the belief that the causal fungus could survive from one growing season till the next on debris left in the field from a previous diseased crop. To test this hypothesis, diseased leaves were collected in the fall and stored out of doors in wire baskets. These were examined at various times during the winter to determine if the frog-eye fungus was still alive. On February 27, many conidia and conidiophores were present on the frog-eye spots on these leaves. The conidiophores were standing in large fascicles and appeared to be growing. When put to germinate in tap water at room temperature, many of the conidia produced 1, 2, and 3 long germ tubes overnight. The stored material was examined again on May 2. Decay had progressed so far that the leaves could not be handled without breaking apart. However, conidia of *Cercospora diazu* could still be found in small numbers, and these appeared to be in a viable condition capable of germination and infection if transferred by wind or other agency to living leaves.

The overwintering of the soybean frog-eye disease on diseased leaves was further tested in the following way: In October 1928 approximately a bushel of diseased leaves were gathered up from the ground beneath soybean plants. These leaves were stored in an orange crate out of doors under some tall shrubbery. On August 20 of the following year, seed of the Otootan variety of soybean were sowed in a garden in which soybeans had not grown in many years and which was nearly 1 mile from the nearest soybean field. The seed used in sowing this garden were harvested the previous season from a variety plot that had been inspected and observed to be free of frog-eye. When the seed was sowed on August 20, the overwintered diseased leaves were scattered about over a part of the bed. Although occasional light rains occurred, the weather remained comparatively dry and was not judged to be particularly favorable for development of fungi in the diseased leaves lying on the ground where they were subject to

rather rapid drying in the hot summer days. Conditions for sporulation of the parasite on the old leaves became more favorable, however, after the seedlings developed sufficient foliage to shade the ground. The first lesions were found on September 26. These were on the lower foliage on the part of the bed over which the diseased leaves had been scattered. Later the disease spread to all parts of the bed.

There still remains the possibility that in this test the plants became infected from wind-blown spores from a diseased field approximately 1 mile away. This seems improbable, however, in view of the following facts: (1) The disease appeared first on the part of the bed to which the overwintered leaves had been applied; (2) the disease did not appear on the varieties Herman and Virginia which had been growing for a much longer period in an adjoining garden; and (3) the disease accumulated comparatively less rapidly in Otootans growing for the entire season less than one tenth mile from the field in which spores were being produced, and between it and the garden in which this test was made. It is highly probable that the diseased leaves saved from the previous season served as the source of inoculum for the beans in the garden test.

On March 26 an examination was made of old stems and pods left in the field after the harvest of a diseased crop of Otootan soybeans in the previous fall. Conidia were readily found on old lesions on both pods and stems. The conidiophores were present in clusters of several to many short individuals. Most of them had at that time formed only one conidium, but many of them were growing forward as they characteristically do before the formation of a second conidium. In size, shape, and septation the conidia found on these overwintered lesions were like those previously observed on diseased leaves. At this time numerous soybean seedlings were coming up in this field from seed which had fallen to the ground when the beans were harvested in the fall. Some of these had unfolded their first leaves—the unifoliate pair. No lesions could be found on these leaves at this time—March 26—but when the field was examined 2 weeks later young frog-eye lesions were present on leaves of approximately 0.5 percent of the seedlings. Later observations could not be made because the field was plowed up at this time. Doubtless some of these germinating seed had come from infected pods, and it may be that the infections present on the leaves of some of the seedlings arose from primary seed infection. However, no seedlings were found, after careful examination, on which there was any evidence, such as growth of the fungus on cotyledons, stem, or seed coat, that the leaf infection had arisen from seed infection. On the other hand, it seemed far more likely that the infection on these seedlings had arisen from conidia produced on overwintered debris from the previous diseased crop.

It often happens, that in plowing the soybean stubble, the diseased stems are only partly turned under, many of them sticking out of the soil between furrows. This is likely to occur when the old stems are so long as to accumulate under the plow beam and slip out in large bundles. In such fields one may often find the frog-eye fungus sporulating in late March or early April. Numbers of volunteer seedlings are also often present that have frog-eye lesions on their first-formed leaves.

A small field in which a badly diseased crop of soybeans had grown in 1928 was divided into two portions that were plowed up at different

times. Numerous lesions had been present on leaves, stems, and pods. The beans were harvested in late October, the stems, pods, and leaves being left on the land. Plot 1 was broken with a turning plow on December 1 and plot 2 on April 25. In this operation the diseased stems, pods, and leaves were turned under, not completely, but very much more so than is accomplished in the usual plowing operation. On May 15 both plots were thoroughly disked and planted, Otootan seed from a disease-free field being used. The disking destroyed the volunteer seedlings which had come up previous to planting on May 15. The beans were planted in rows, and notes on the presence of frog-eye were taken from time to time as the season advanced.

The first notes were taken on June 13, at which time the plants, having shed their cotyledons, possessed two fully expanded trifoliate leaves in addition to the usual pair of unifoliate leaves and a third folded trifoliate leaf. On plot 1, at this time, it was estimated that 10 percent of the plants had frog-eye lesions on their unifoliate leaves, there being usually 1, but sometimes 2, 3, and 4 spots per individual leaf. A single lesion was found on a trifoliate leaf. A very few volunteer plants had come up tardily between the rows, apparently from the slow germination of seed having hard coats, but none of these were diseased. Several scattered plants which obviously had germinated before the land was disked for planting and had not been killed by this operation, were present in the plot. These could be identified readily by their larger size. Only one of these showed frog-eye lesions. This one plant could not have been responsible for any considerable proportion of the lesions on the other plants. It seems a safe conclusion that the disease on most of the plants which came from disease-free seed planted on May 15 resulted from spores produced on the overwintered debris.

Plot 2, which had been turned under in April, was separated from plot 1 by 20 rows of corn. Careful observation indicated that there were not appreciably more plants showing infection of unifoliate leaves on plot 2 than on plot 1. Lesions were found, however, on several trifoliate leaves of plot 2, as compared with only one on plot 1, but the difference in number seemed so meager as to be without significance. Later observations revealed no difference in amount of disease on the two plots. By August 15 all plants were infected, 75 percent of the leaves showing one or more lesions. The test demonstrates satisfactorily that the debris from diseased fields may harbor the disease till the following spring and that this debris is not plowed under sufficiently well in the usual farm operations to prevent recurrence of the frog-eye disease even though disease-free seed is used.

FROG-EYE FUNGUS ON DISEASED SEED

The presence of the frog-eye fungus on and within the seed coat of beans taken from diseased pods has been amply demonstrated in the foregoing paragraphs. Evidence that the use of diseased seed is responsible for the appearance of frog-eye in new fields or new communities is found in the following tests: Although observations were being made over a period of several years in connection with the study of other soybean diseases, the frog-eye disease was not seen in the field in the vicinity of Raleigh until 1927. In that year, seed of the

Biloxi variety, produced in 1926 in a distant part of the State in a field known to be infested with frog-eye to a moderate degree, was planted at Raleigh. Seed of the Laredo variety likewise obtained from a distant part of the State was also planted at Raleigh. A portion of each kind of seed was treated with chemical disinfectants, the remaining portion being left untreated as a check on the treatments. Plantings were made May 25. No frog-eye lesions could be found on July 6 or August 3. On August 17 a small number of lesions were found scattered at irregular distances along the check rows of both Biloxi and Laredo, while none was found at this time on rows which had been planted to treated seed, although there were 1,900 feet of treated rows, as compared with 1,000 feet of untreated rows. It seems obvious that the disease originated in this field from infected seed. If it had been introduced by means of spores carried long distances by the wind, lesions should have been equally prevalent on all rows in this test. Observations indicate that, at the early date at which the first infections were noted in this test, the fungus had not produced a sufficient quantity of spore material to account for any but the most meager aerial spore population.

In another test, Otootan seeds harvested from diseased plants and here designated as lot 1 were planted at one side of a field which had been in cotton the previous year. Lot 2, which was planted on the opposite side of the field, consisted of Otootan beans from a different source, but selected with the expectation that they were disease free. This planting was visited for taking notes on August 6, approximately 3 months after the beans had been planted. At that time frog-eye was found at scattered locations along the rows planted with the infected seed (lot 1), but none was found on the rows planted with disease-free seed (lot 2). On the plants of lot 1, the lesions were rather numerous at a few locations along the rows as one would expect if the first secondary infections had resulted from the germination of diseased seed at these particular places. At these places there were many leaves with several spots per leaf, and a majority of the lesions were on leaves at the middle or bottom of the plants. Between these centers of dense infestation there was a sparse distribution of lesions on upper leaves. These lesions were initiated presumably from spores produced at the places of primary seedling infection. Since in this test frog-eye occurred only on plants from infected seed of lot 1 and not on plants from the seed of lot 2 which were equally exposed to possible wind-borne infection, it is believed that the disease originated from fungi carried to the field in the seed of lot 1.

The exact course taken by the frog-eye fungus in passing from the infected seed to the foliage of the plant which grew therefrom has not been satisfactorily determined. It might be expected that the fungus would grow in and sporulate on the tissues of the expanded cotyledons of the seedling, the spores passing from the cotyledon via the air to the unifoliate leaves or the stem and finally to trifoliate leaves formed later. However, the writer has never been able to find cotyledonary lesions unquestionbly those of the frog-eye fungus on plants in the field. Isolations have been made from suspected lesions; but none has yielded the frog-eye fungus, while little or no difficulty has ever been experienced in isolating this fungus from leaf, pod, or stem infections. When infected seed is germinated on agar in test tubes, the fungus often becomes visible only at places where the seed has touched the agar. Usually in test tubes the seed coat does

not shed off the seed leaves but often clings to one or both of them as they are elevated into the air by the elongating hypocotyl. The epicotyl may elongate, pushing the plumule out from between the cotyledons which are held rather firmly together by the adhering seed coat. The frog-eye fungus often grows out and sporulates on the surface of infected seed coats held in this position. On a few such seedlings in large test tubes the fungus has been observed to fruit first on the uplifted seed coat and later to form typical reddish elongated lesions on the hypocotyl, presumably as the result of spores dropped from the seed coat above. Lesions have been produced on hypocotyls by rubbing conidia on them with an inoculating needle. On a few seedlings germinating in test tubes the mycelium of the frog-eye fungus has been observed to grow from the seed coat onto the cotyledonary tissue, but it has not exhibited any ability to parasitize these tissues to the extent of withdrawing nourishment from and sporulating on them. Reasoning from the behavior of the fungus on seedlings growing in large test tubes and from the fact that cotyledonary lesions have not been identified in the field, one is led to the assumption that in the field the fungus sporulates on the seed coat of infected seeds and the spores are then transported from there to the true leaves by the wind or other agencies. As the cotyledons are pushed upward through the soil by the elongating hypocotyl, the seed coat is pulled off the great proportion of the seedlings and left in the soil. Only a small proportion of the seed coats ever come above the ground and attain an elevation where spores formed on them are likely to be picked up by wind. This circumstance may account for the observed tardiness with which frog-eye arises and the slowness with which infestation becomes conspicuous when it originates from diseased seed. The writer has observed that field infestation reaches discernible proportions later in the summer when the disease arises from infected seed than when its source is in debris left on the field from a diseased crop. The percentage of infected seeds effective in initiating infections in the new crop is probably greatly reduced by the natural stripping off of the seed coat while still in the ground. This is a fortunate circumstance, for, otherwise, field infestations arising as they usually do from infected seed would reach harmful proportions earlier in the summer and thus result in greater harm to the plants.

SUMMARY

Infections by the frog-eye fungus (*Cercospora diazu* Miura) of soybean have been observed in large numbers not only on leaves, as noted in a previous publication, but also on stems, pods, and seeds.

The causal organism has been isolated from frog-eye lesions on leaves, stems, pods, and seeds, and reciprocal inoculations have been made. The cultures from these different sources were alike, and positive infections were obtained in sufficiently large numbers to justify the conclusion that the lesions on leaves, stems, pods, and seeds were caused by one and the same species, namely, the frog-eye fungus, *Cercospora diazu*.

Cauline lesions appear in abundance only in the late part of the growing season when stem tissues are ripening. The diseased areas are elongated and may be flattened or slightly sunken. The lesion is some shade of red when young, but changes to brown, then smoke gray with age. Black stromata of pin-point dimensions are often

visible in large numbers on old lesions, and the diseased area may become black because of more or less pronounced stromatic development of dark-colored mycelium in the invaded tissue.

In stem lesions, the mycelium of the parasite grows chiefly in the cortex, being confined there by the presence of a pericyclic layer of sclerenchyma between cortical and phloem tissues. The phloem and cambium are usually damaged, however. Sometimes this is caused by actual invasion of these tissues by the fungus, but more often no mycelium is present in them, the obvious injury being due presumably to diffusion of toxic materials from the necrotic portion of the adjacent cortex.

Pod lesions are usually quite round, somewhat sunken, and vary in diameter from 1 to 4 mm. Young lesions on green pods are brown, often with a tint of red. A uniform brown color may persist as the pod matures, but usually on dry, ripe pods the diseased area appears as a light-brown spot encircled by a dark-brown ring. Conidiophores and conidia are often present.

Mycelium grows completely through the pod wall entering the thin white membrane which lines the pod and closely invests the seeds. Portions of this infected membrane often adhere to the seed coat and thus serve, but not invariably, to designate the individual seeds which are infected.

No definite lesion or marked discoloration is produced on the portion of the seed coat which develops in contact with a pod-wall lesion. However, there is often at this place a slight depression in the seed coat and a lack of the smoothness and luster characteristic of the coats of healthy seeds. This roughened, depressed area is a less conspicuous but more infallible sign of seed infection than is the presence of bits of pod-wall lining adhering to the seed coat.

Seed-treatment tests show that the frog-eye fungus makes intimate contact with a large proportion of the seeds which develop in touch with pod-wall lesions. In a large percentage of such seeds the fungus is so superficial as to be killed by seed disinfectants. It is actually in the seed-coat tissues and beyond the reach of disinfectants in a relatively few seeds. Infection of the embryo occurs rarely, if at all.

It is possible by inspection of a given seed lot to determine whether or not any considerable proportion of the pods from which the seed came were infected. Experience indicates that where as many as 10 percent of the seeds show signs of the frog-eye disease, practically 100 percent of the leaves of the plants which produced the seed were infected to the extent that considerable defoliation occurred.

The frog-eye fungus has been found still viable in potato-agar test-tube cultures 519 days old.

This fungus overwinters on diseased leaves and stems. The disease was initiated experimentally in an uninfested test plot by scattering diseased leaves over the ground. It has been observed to develop when disease-free seed was sown on land that bore a diseased crop the previous season. Breaking the infested stubble in the fall did not prevent or even appreciably retard disease development the next season. Diseased stems cannot be turned under sufficiently well to prevent recurrence of the disease if soybeans follow soybeans without an intervening crop.

The use of diseased seed is a means of introducing the disease into new fields and new communities.

VARIETAL DIFFERENCES IN COTTON BOLL SHEDDING AS CORRELATED WITH OSMOTIC PRESSURE OF EXPRESSED TISSUE FLUIDS [1]

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INTRODUCTION

Results previously reported [2] indicate an inverse relationship between the shedding of young cotton bolls of the Acala variety and the osmotic pressure of the expressed leaf fluids. Low osmotic pressures were found to be followed by high shedding and relatively high osmotic pressures by low shedding. Anatomical studies of stem tissues indicated that during periods of low osmotic pressure plant food was used largely to build vegetative tissues to the detriment of fruiting parts. Conversely, when osmotic pressures were relatively high vegetative growth was depressed and fruiting parts were supplied with adequate plant food, resulting in low shedding. Extremely high osmotic pressures induced by severe drought were followed not only by suppressed vegetative growth but also by intense shedding.

The findings previously reported with the Acala variety have been corroborated and additional data obtained on other cotton varieties, boll shedding in which does not always respond to osmotic-pressure variations in quite the same manner as in the Acala variety. Varietal differences in boll shedding have been investigated with the idea that differences between the osmotic pressures in the leaves and in the adjoining bolls of the same variety might account for heritable differences in shedding. If the osmotic pressures were materially higher in the leaves than in the adjoining bolls the bolls would not always be able to compete with the leaves for the water in the tissue fluids, and high shedding would follow. It was thought that the differences in osmotic pressure between leaves and bolls in the Pima variety of cotton, in which shedding is very low, seldom reaching more than 25 or 30 percent, might be found to be very small as compared with those in Acala, for example, in which boll shedding often reaches 70 or 80 percent.

METHODS

In the first experiment a comparison was made between the Pima and Acala varieties in the matter of boll shedding as affected by osmotic-pressure differences in the tissue fluids of the leaves and bolls. The plants were grown in 1926 on adjacent plots at the Salt River Valley Experiment Farm and given similar treatment through-

[1] Received for publication Sept. 20, 1933; issued March, 1934.

[2] Hawkins, R. S., MATLOCK, R. L., and HOBART, C. PHYSIOLOGICAL FACTORS AFFECTING THE FRUITING OF COTTON WITH SPECIAL REFERENCE TO BOLL SHEDDING. Ariz. Agr. Expt. Sta. Tech. Bull. 46, pp. 361–407, illus. 1933.

out the season. The soil is a fertile clay loam, and excellent plant growth was obtained with both varieties. Climatological measurements, including atmospheric and soil temperature, rainfall, cloudiness, wind movement, and evaporation, were obtained in connection with another experiment on water variations in the leaves of these two varieties of cotton. These data, together with soil-moisture data, are recorded elsewhere [3] and will not be repeated here since this experiment is concerned primarily with differences in shedding as influenced by variations in osmotic pressure and only incidentally with the factors that affect changes in osmotic pressure.

The shedding behavior of the 2 varieties was ascertained by daily tagging all flowers on 25 adjacent plants in each plot and then noting the number of tagged bolls that were shed each day. Leaves and day-old bolls were collected daily at 8 a.m. from July 28 to August 25 for the determinations of osmotic pressure. The leaves used were those adjacent to the bolls collected. Two men made the collections; one collected material from the Acala variety while the other made parallel collections from the Pima variety. Seven tubes of leaves and seven tubes of bolls were collected each day from each variety. The tubes were placed in an ice-salt freezing mixture within a few minutes after the daily collections were completed. After several hours of freezing, the material while still in the tubes was removed from the freezing mixture and allowed to thaw for a short time. The juices were then expressed by means of a fruit press. Harris [4] employed a similar press in his work with cotton when a press equipped with a pressure gage was not available, a procedure not altogether desirable but at times unavoidable.

The freezing-point method of determining the atmospheres of osmotic pressure, as described by Dixon and Atkins,[5] Gortner and Harris,[6] and Lawrence and Harris [7] was used.

In 1927 a second experiment was made. In this experiment shedding and osmotic-pressure variations were investigated with four varieties of cotton, viz: Pima, Acala, an F_1 cross between Acala and Pima, and a small-boll American Indian variety known as Sacaton Aboriginal. These four varieties exhibit wide variations in shedding behavior. The plots were located on the Salt River Valley Experiment Farm and soil conditions other than moisture were approximately similar to those in experiment 1. The technic for osmotic-pressure determinations was not varied from that employed in experiment 1 except that 6 tubes of leaves and 6 tubes of bolls of each variety were used for the daily determinations.

RESULTS AND DISCUSSION

EXPERIMENT 1

The shedding behavior of the Acala variety was quite consistently correlated with osmotic pressures in the leaves and bolls, being high

[3] Hawkins, R. S., Matlock, R. L., and Hobart, C. (See footnote 2.)

[4] Harris, J. A., Lawrence, Z. W., Hoffman, W. F., Lawrence, J. V., and Valentine, A. T. The tissue fluids of Egyptian and upland cottons and their F_1 hybrid. Jour. Agr. Research 27: 267–328, illus. 1924.

[5] Dixon, H. H., and Atkins, W. R. G. Osmotic pressures in plants. Methods of extracting saps from plant organs. Roy. Dublin Soc. Sci. Proc. 13: 422–433. 1913.

[6] Gortner, R. A., and Harris, J. A. Notes on the technique of the determination of the depression of the freezing point of vegetable saps. Plant World 17: 49–53. 1914.

[7] Lawrence, J. V., and Harris, J. A. The extraction of sap from plant tissues by pressure. Biochem. Bull. 5: 139–142, illus. 1916.

when osmotic values were low, and low when osmotic values were high (fig. 1). These results are in accord with those reported previously.[8] Two irrigations were given during the portion of the fruiting period under investigation, that is, on August 4 and 19. These dates coincide with the two peaks in the osmotic-pressure variations. Thus the irrigations indirectly influenced the shedding behavior of the plants.

In the Acala variety the osmotic pressures in the bolls were always somewhat lower than in the leaves. The same was true of the Pima variety (fig. 2) with the exception of the material collected on August 18 and 19, when the osmotic pressures in the bolls were slightly higher than in the leaves. However, the results do not appear to substantiate the assumption that wide differences between osmotic

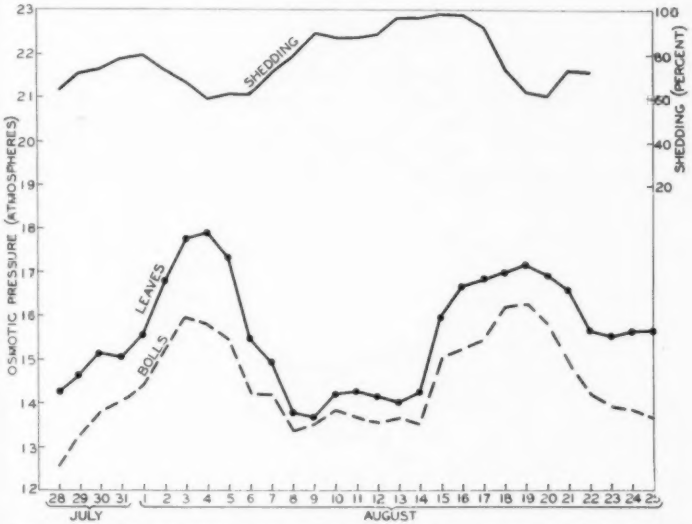

FIGURE 1.—Shedding percentages and osmotic pressures in the leaves and bolls of Acala cotton.

pressures in the leaves and bolls on the same plants at any given date materially increase shedding. For example, shedding was at a minimum with bolls whose flowers opened on August 4, although osmotic pressures in the bolls and leaves were at wider variance than at other times when shedding was higher (fig. 1). Many similar examples which refute the foregoing assumption are evident from a study of the shedding and osmotic-pressure curves in figures 1, 2, 4, and 5.

The curves for Pima, presented in figure 2, show little if any correlation between shedding and osmotic pressure. Shedding is very low in this variety, however, seldom reaching more than 30 percent on any one day. In this investigation the bolls shed by the Pima plants averaged between 17 and 18 percent. Most of the

[8] HAWKINS, R. S., MATLOCK, R. L., and HOBART, C. (See footnote 2.)

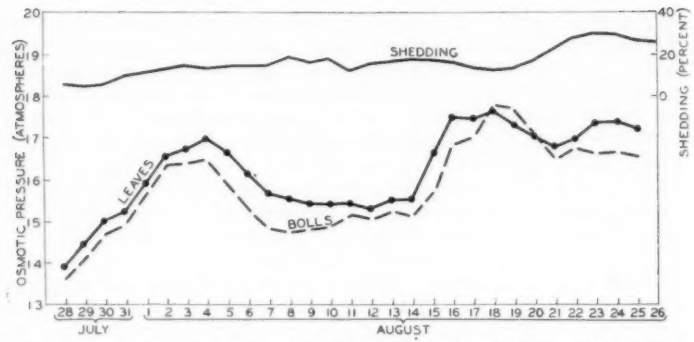

FIGURE 2.—Shedding percentages and osmotic pressures in the leaves and bolls of Pima cotton.

shedding of the Pima bolls may have been due to insect damage or to a lack of effective pollination, or both. With a heavy-shedding variety like Acala, which shed 78 percent during this same period, the same amount of insect damage or imperfect pollination would be greatly overshadowed by other influential factors such as asmotic pressure of the tissue fluids.

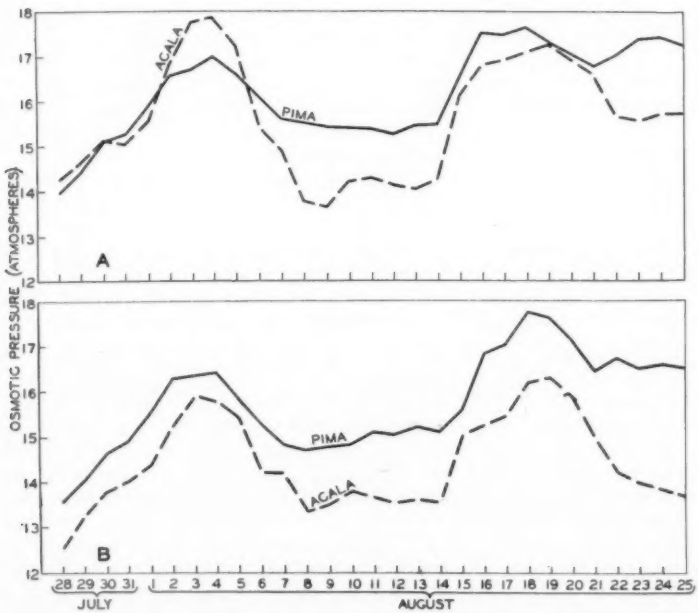

FIGURE 3.—Osmotic pressure in the leaves (*A*) and bolls (*B*) of Pima and Acala cottons.

It has been shown previously [9] that high osmotic pressures are correlated with decreased shedding in the Acala variety. The osmotic pressures were always materially higher in the Pima bolls than in the Acala bolls (fig. 3, *B*). A similar situation prevailed in the leaves of the two varieties with few exceptions (fig. 3, *A*). These higher osmotic values in the Pima plants may account for the limited amount of boll shedding. This assumption is supported further by data obtained in connection with experiment 2.

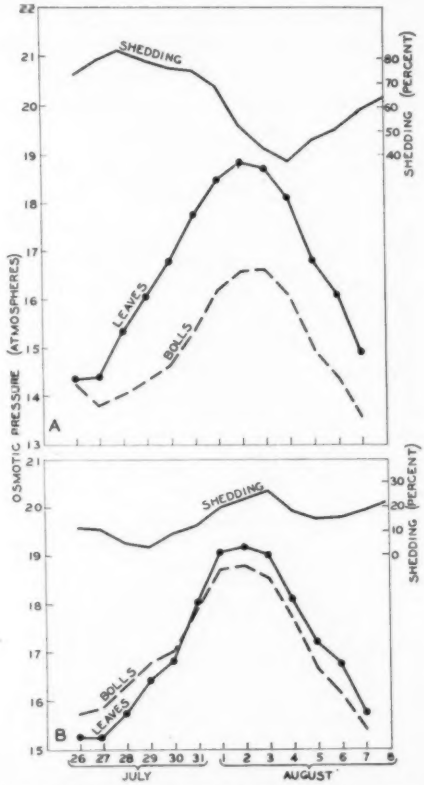

FIGURE 4.—Shedding percentages and osmotic pressures in the leaves and bolls of (*A*) Acala and (*B*) Pima cottons.

EXPERIMENT 2

Osmotic pressures and boll shedding in the Acala variety again were correlated (fig. 4, *A*). The characteristic wide spread between the osmotic pressures in the leaves and in the bolls was also exhibited. Whether this wide spread has any significance in relation to shedding is not known. No correlation between osmotic pressures and shedding is indicated in the Pima variety, which coincides with

[9] HAWKINS, R. S., MATLOCK, R. L., and HOBART, C. (See footnote 2.)

the results obtained in experiment 1 (fig. 4, *B*). A good correlation is shown for the first-generation hybrid of Pima and Acala at the period of high osmotic pressure and immediately following this peak, although little if any is shown for the rest of the period under investigation (fig. 5, *A*). The osmotic pressure values for the bolls of the Pima × Acala hybrid were intermediate between those for the bolls of the two parents, and the correlaton between osmotic pressure and shedding in the hybrid conforms to expectation. The Sacaton Aboriginal, a variety which flowers profusely, had consistently low osmotic values. Variations in shedding were not great and the shedding curve shows a good negative correlation with osmotic pressure (fig. 5, *B*).

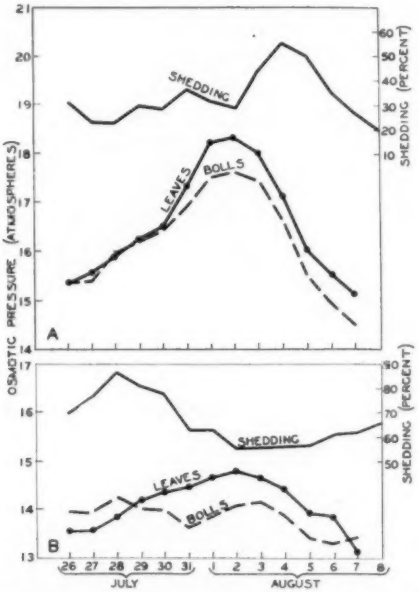

FIGURE 5.—Shedding percentages and osmotic pressures in the leaves and bolls of (*A*) an F_1 Pima × Acala hybrid and (*B*) of Sacaton Aboriginal cotton.

The shedding curves of the four varieties have been superimposed in figure 6. The superimposed curves showing the osmotic pressures of the bolls and of the leaves are shown in figure 7. A comparison of the graphs demonstrates that the order of arrangement of the four varieties on the basis of shedding behavior is the reverse of their arrangement on the basis of osmotic pressure in the bolls. Pima had the lowest shedding and the highest osmotic pressures, while Sacaton Aboriginal had the highest shedding and the lowest osmotic pressures. The other two varieties were intermediate both in shedding and in osmotic pressure. These results are entirely in accord with those obtained in experiment 1 and with the data reported previously.[10] The osmotic pressures in the leaves of

[10] HAWKINS, R. S., MATLOCK, R. L., and HOBART, C. (See footnote 2.)

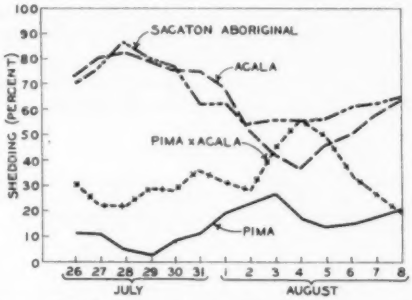

FIGURE 6.—Shedding percentages of four varieties of cotton.

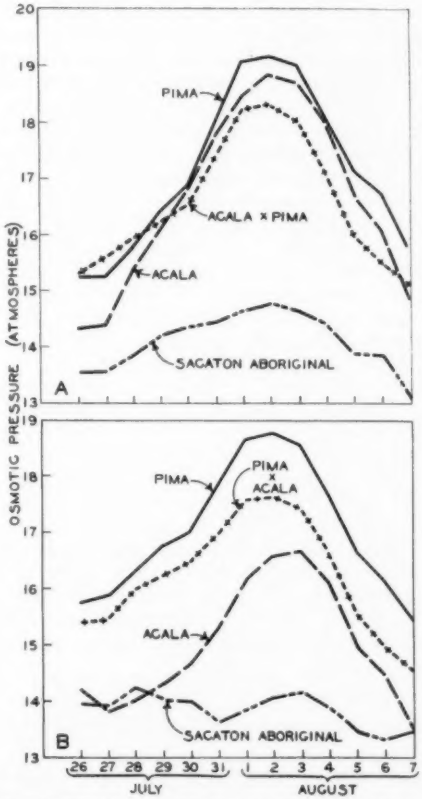

FIGURE 7.—Osmotic pressures in the leaves (*A*) and bolls (*B*) of four varieties of cotton.

Pima are the highest and those in the leaves of Sacaton Aboriginal the lowest, but the order is reversed with the two intermediate varieties. Possibly this indicates that osmotic pressures in the bolls are more directly correlated with shedding behavior than are the osmotic values in the leaves, although both undoubtedly are involved.

SUMMARY

The shedding of young Acala cotton bolls was definitely correlated with the osmotic pressures of the expressed tissue fluids of the leaves and day-old bolls.

The daily changes in boll shedding and osmotic pressures show no correlation in the Pima variety. The extremely low shedding coupled with comparatively high osmotic pressures may account for the lack of correlation.

The spread between the osmotic pressures in the leaves and in the bolls does not seem to be correlated with shedding.

The superimposed shedding curves of four varieties differing widely in shedding behavior show a reverse arrangement as compared with the superimposed curves showing osmotic pressures in the bolls, which indicates a good negative correlation between shedding and osmotic pressure in the bolls.

Boll shedding is somewhat more closely correlated with osmotic pressure in the bolls than with osmotic pressure in the leaves.

BORDER EFFECT IN IRRIGATED PLOTS OF MARQUIS WHEAT RECEIVING WATER AT DIFFERENT TIMES[1]

By D. W. ROBERTSON, *associate agronomist*, and DWIGHT KOONCE, *assistant agronomist, Colorado Agricultural Experiment Station*

INTRODUCTION

Extensive data are available on the border effect in plots in which different varieties of plants have been grown under humid conditions. Little study has been made, however, of the border effect on the same crops grown under irrigation. This paper presents the results of experiments made to determine the border effect on Marquis wheat grown in plots irrigated at different stages, and shows the relationship of the yields when various numbers of border rows are included.

MATERIALS AND METHODS

The plots were located on land that had been fallowed the previous season. The wheat was sown with a 16-disk drill, and after sowing plots approximately one five-hundredth of an acre were measured off and diked.

The plots were laid out end to end, a dike about 1 foot wide separating each plot. In the fall one plot was diked and irrigated with 6 inches of water. The basin method of irrigation was used.[2] After the soil had dried on the surface and before snow had fallen, the dikes were leveled with the rest of the series. This prevented any unequal drifting of snow over the area used. In the spring after planting, a second plot received 7 inches of water at germination, and the other plots received 1 inch of water to insure an even germination. The third plot received a 6-inch irrigation at jointing, or just before heading. All of the plots, therefore, received a total irrigation of 7 inches as well as the seasonal rainfall. There were three plots for each treatment each year. At harvest time 1 foot was cut from the end of each row, leaving a plot 10 feet long. The plots were harvested as follows: Outside border rows 1 and 16 were harvested separately with grass shears; rows 2 and 15 and 3 and 14 were similarly harvested; rows 4 to 13, inclusive, were harvested together, making the 10 center rows. All of the bundles were wrapped in cloth and cured under cover. A modified Cornell thresher was used for threshing all bundles. Total weights of grain and straw, uncleaned weight of grain, and cleaned weight of grain were taken. The difference between the cleaned weight and the total weight is considered as straw yield. The plots were systematically distributed.

The experiment as outlined was carried on for 4 years, 1928 to 1931, inclusive.

[1] Received for publication Aug. 2, 1933; issued March, 1934. Contribution from the Department of Agronomy, Colorado Agricultural Experiment Station.

[2] KEZER, A., and ROBERTSON, D. W. THE CRITICAL PERIOD OF APPLYING IRRIGATION WATER TO WHEAT. Jour. Amer. Soc. Agron. 19: 93. 1927.

RAINFALL

Rainfall, which varied during the different seasons, affected the total yield. In the dry season of 1931 the yield for all plots was smaller than in any other year of the experiment.

The rainfall data for 1928 to 1931 are given in table 1.

TABLE 1.—*Rainfall* [a] *at Fort Collins, Colo., 1928–31*

Month	1928	1929	1930	1931	Month	1928	1929	1930	1931
	Inches	*Inches*	*Inches*	*Inches*		*Inches*	*Inches*	*Inches*	*Inches*
January	0.26	0.21	0.45	0.00	August	.69	2.35	5.36	.75
February	.52	.70	.70	1.26	September	.09	2.13	.16	.51
March	1.38	1.78	.56	.41	October	1.50	.99	.35	1.00
April	1.02	2.37	.49	1.07	November	1.15	.93	.70	.63
May	3.01	.92	3.92	2.93	December	.06	.09	.14	.18
June	2.95	.64	1.50	1.46					
July	.79	.46	1.04	.05	Total	13.42	13.57	15.37	10.25

[a] The rainfall records for January, February, March, November, and December were obtained from R. E. Trimble, assistant irrigation investigator.

DATES OF PLANTING, IRRIGATION, AND RIPENING

Marquis wheat was planted on April 9, 1928; April 8, 1929; April 7, 1930; and April 6, 1931. The dates of irrigation and the dates of ripening of the grain on the various plots are given in table 2. This table shows little, if any, difference in the date of maturity of the grain, in any one year, on the plots treated.

TABLE 2.—*Dates of applying irrigation water to plots, and of ripening of the grain on the variously treated plots, 1928–31*

DATES OF APPLYING IRRIGATION WATER TO PLOTS

Treatment	Data for plots planted on—			
	Apr. 9, 1928	Apr. 8, 1929	Apr. 7, 1930	Apr. 6, 1931
Irrigation in fall	Sept. 9, 1927	Sept. 10, 1928	Oct. 1, 1929	Oct. 6, 1930
Irrigation at germination	Apr. 14, 1928	Apr. 20, 1929	Apr. 14, 1930	Apr. 13, 1931
Irrigation at jointing	June 7, 1928	June 18, 1929	June 10, 1930	June 15, 1931

DATES OF RIPENING OF THE GRAIN

Irrigation in fall	Aug. 8, 1928	July 30, 1929	July 22, 1930	July 24, 1931
Irrigation at germination	do	do	do	Do.
Irrigation at jointing	do	do	do	July 26, 1931

YIELD DATA

In studying the border effect, 10 center rows are considered as a unit of 100 percent. The 12 rows are made up of the 10 center rows and the inside border rows (3 and 14). The 14-row plots are made up of the 10 center rows, the inside border rows, and the middle border rows (2 and 15). The 16-row plots include the 10 center rows, the inside border rows, the middle border rows, and the outside rows (1 and 16). Plots of 10, 12, 14, and 16 rows are used in the discussion of border effect. The arrangement of the rows is shown in figure 1.

The yield data show considerable variation between the different years. When the yields of the outside rows, 1 and 16, are compared with those of the 10 center rows, a considerably higher percentage

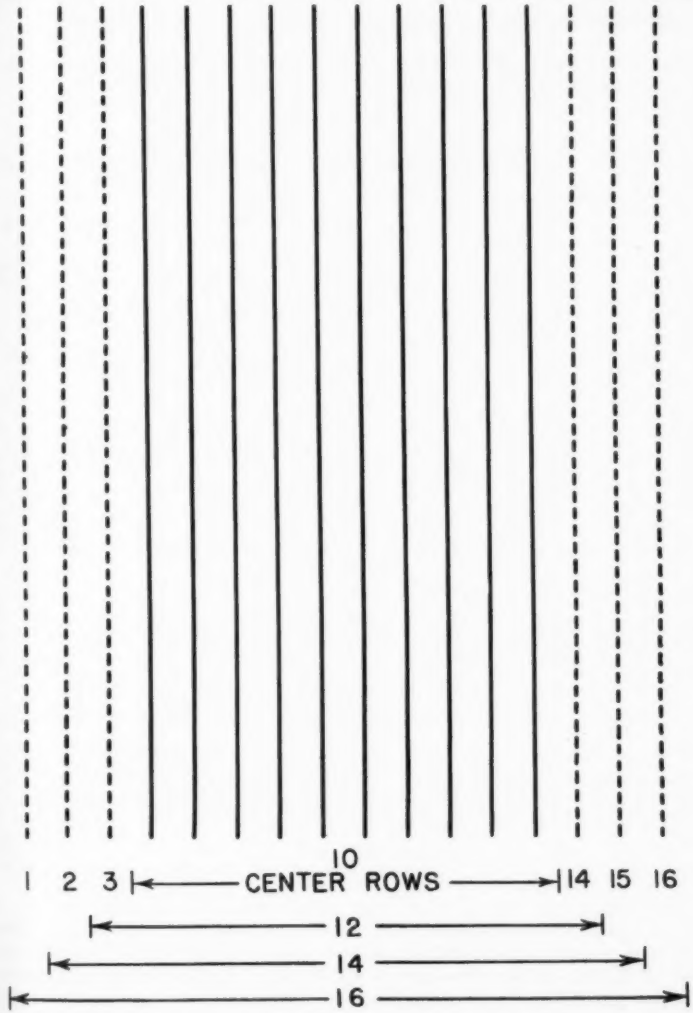

FIGURE 1.—The arrangement of rows in the differently treated plots used in the computation of yield data.

value is obtained than when the yield of the entire plot (16 rows) is compared with the yield of the 10 center rows. The same is true of rows 2 and 15, but to a lesser degree. The average yield for each plot of 10, 12, 14, and 16 rows is given in table 3. The table shows that while the yield varied in different years the yield in percentage

when compared with the fall-irrigated plots as 100 percent holds the same relative position for plots of 10, 12, 14, or 16 rows.

The variation in straw yield was about the same as in grain yield, except that yields of straw were slightly smaller for the plots irrigated at germination and slightly larger for those irrigated at jointing.

TABLE 3.—*Yields from different numbers of border rows harvested from plots of Marquis wheat receving irrigations at different times, 1928–31*

Year	Grain yield from plots irrigated at time indicated						Straw yield from plots irrigated at time indicated					
	Fall		Germination		Jointing		Fall		Germination		Jointing	
	Grams	*Per-cent*	*Grams*	*Per-cent*	*Grams*	*Per-cent*	*Grams*	*Per-cent*	*Grams*	*Per-cent*	*Grams*	*Per-cent*
10 CENTER ROWS												
1928	1,384	100	1,527	110	1,533	111	2,892	100	2,785	96	3,433	119
1929	1,020	100	884	87	1,434	141	1,667	100	1,459	88	2,470	148
1930	1,180	100	1,144	97	1,390	118	2,290	100	2,004	88	3,289	144
1931	825	100	757	92	1,111	135	1,447	100	1,211	84	2,075	143
Average	1,102	100	1,078	98	1,367	124	2,074	100	1,865	90	2,819	136
12 ROWS												
1928	1,647	100	1,839	112	1,815	110	3,408	100	3,330	98	4,075	120
1929	1,226	100	1,090	89	1,715	140	2,002	100	1,752	88	2,992	149
1930	1,389	100	1,350	97	1,674	121	2,714	100	2,372	87	3,942	145
1931	994	100	910	92	1,348	136	1,733	100	1,489	86	2,539	147
Average	1,314	100	1,297	99	1,638	125	2,464	100	2,236	91	3,387	137
14 ROWS												
1928	2,023	100	2,238	111	2,229	110	4,154	100	4,084	98	4,955	119
1929	1,535	100	1,383	90	2,114	138	2,501	100	2,214	88	3,695	148
1930	1,718	100	1,618	94	2,063	120	3,337	100	2,844	85	4,798	144
1931	1,191	100	1,097	92	1,596	134	2,107	100	1,781	85	3,009	143
Average	1,617	100	1,584	98	2,001	124	3,025	100	2,731	90	4,114	136
16 ROWS												
1928	2,551	100	2,900	114	2,870	113	5,237	100	5,212	100	6,287	120
1929	2,007	100	1,831	91	2,707	135	3,253	100	2,958	91	4,664	143
1930	2,210	100	2,006	91	2,555	116	4,213	100	3,524	84	5,768	137
1931	1,387	100	1,336	96	1,861	134	2,572	100	2,302	90	3,604	140
Average	2,039	100	2,018	99	2,498	123	3,819	100	3,499	92	5,081	133

By comparing the yield of the 10 center rows alone with the 10 center rows plus the inside border rows (12 rows), the 10 center rows plus the inside and middle border rows (14 rows), and the entire plot of 16 rows, the increase in percentage yield over the 10 center rows is found to be gradual and is approximately the same for all treatments. While the yearly yield of grain varies from 825 to 1,384 g, the percentage increase for the different number of border rows is approximately the same. However, the effect of the outside border rows in the dry season of 1931 is slightly less than in the other 3 years. It varies only 6 percent between treatments. Table 4 gives the average yearly yield for the 10 center rows and for the 10 center rows plus 2, 4, and 6 border rows.

The increase in yield of both straw and grain is gradual. The average figures for grain yield range from 119 to 120 percent for the 10 center rows plus the inside border row, 146 to 147 for the 10 center

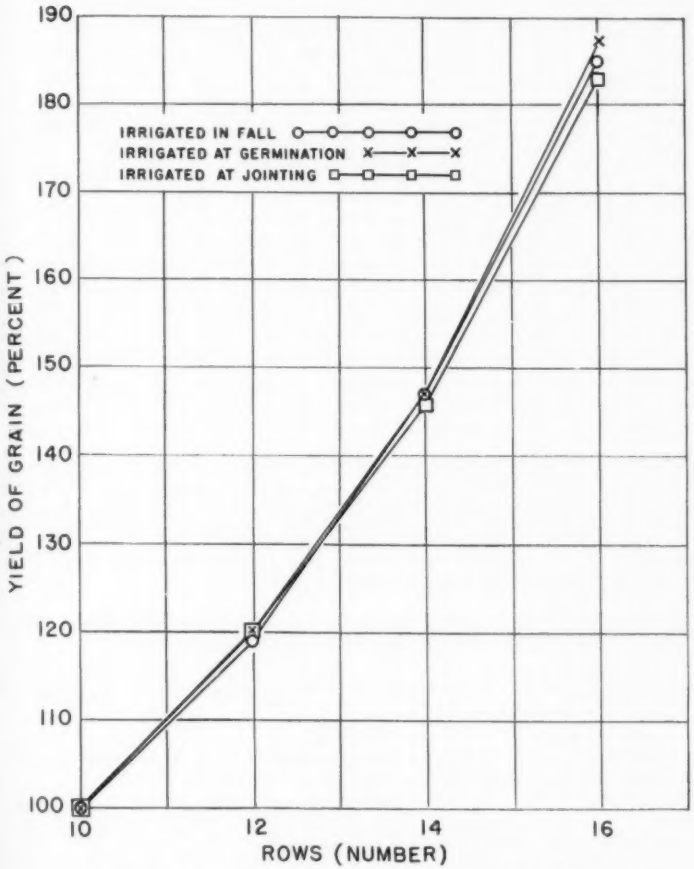

FIGURE 2.—Percentage yield of grain for plots of 10, 12, 14, and 16 rows.

rows plus the inside and middle border rows, and from 183 to 178 percent for the entire plot (fig. 2). A very similar range is found for the straw yield. The time of treatment had no consistent effect on the yield, all treatments having about the same percentage increase for the different number of border rows.

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TABLE 4.—*Yields from 10 center rows and from plots consisting of 10 center rows plus 2, 4, or 6 border rows, 1928–31*

Year	Rows	Grain yield from plots irrigated at time indicated						Straw yield from plots irrigated at time indicated					
		Fall		Germination		Jointing		Fall		Germination		Jointing	
	Number	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*	*Grams*	*Per cent*
1928	10	1,384	100	1,527	100	1,533	100	2,892	100	2,785	100	3,433	100
	12	1,647	119	1,839	120	1,815	118	3,408	118	3,330	120	4,075	119
	14	2,023	146	2,238	147	2,229	145	4,154	144	4,084	147	4,955	144
	16	2,551	184	2,900	190	2,870	187	5,237	181	5,212	187	6,287	183
1929	10	1,020	100	884	100	1,434	100	1,667	100	1,459	100	2,470	100
	12	1,226	120	1,090	123	1,715	120	2,002	120	1,752	120	2,992	121
	14	1,535	150	1,383	156	2,114	147	2,501	150	2,214	152	3,695	150
	16	2,007	197	1,831	207	2,707	189	3,253	195	2,958	202	4,664	189
1930	10	1,180	100	1,144	100	1,390	100	2,290	100	2,004	100	3,299	100
	12	1,389	118	1,350	118	1,674	120	2,714	119	2,372	118	3,942	119
	14	1,718	146	1,618	141	2,063	148	3,337	146	2,844	142	4,798	145
	16	2,210	187	2,006	175	2,555	184	4,213	184	3,524	176	5,768	175
1931	10	825	100	757	100	1,111	100	1,447	100	1,211	100	2,075	100
	12	904	120	910	120	1,348	121	1,733	120	1,489	123	2,539	122
	14	1,191	144	1,097	145	1,596	144	2,107	146	1,781	147	3,009	145
	16	1,387	168	1,336	176	1,861	168	2,572	178	2,302	190	3,604	174
Average	10	1,102	100	1,078	100	1,367	100	2,074	100	1,865	100	2,819	100
	12	1,314	119	1,297	120	1,638	120	2,464	119	2,236	120	3,387	120
	14	1,617	147	1,584	147	2,000	146	3,025	146	2,731	146	4,114	146
	16	2,039	185	2,018	187	2,498	183	3,819	184	3,499	187	5,081	180

There is no consistent increase or decrease in variability of the plots. Table 5 gives the average yield in grams of the fall-irrigated plots and the probable error in percentage of the mean. The probable errors were determined by the deviation-from-the-mean method. There is a variation between different years, but no consistent trend up or down as the number of rows increase.

TABLE 5.—*Comparison of variability in the different fall-irrigated plots as determined by the probable error of the mean, in percentage*

Year	Rows	Grain yield		Straw yield	
		Fall-irrigated plots	Probable error	Fall-irrigated plots	Probable error
	Number	*Grams*	*Percent*	*Grams*	*Percent*
1928	10	1,384	±2.74	2,892	±3.47
	12	1,647	±2.50	3,408	±3.46
	14	2,023	±2.22	4,154	±3.32
	16	2,551	±2.87	5,237	±3.59
1929	10	1,020	±2.28	1,667	±2.56
	12	1,226	±2.24	2,002	±3.42
	14	1,535	±1.82	2,501	±2.96
	16	2,007	±1.60	3,253	±2.79
1930	10	1,180	±2.25	2,290	±2.69
	12	1,389	±2.17	2,714	±2.52
	14	1,718	±1.87	3,337	±2.28
	16	2,210	±1.27	4,213	±1.64
1931	10	825	±1.66	1,447	±1.80
	12	904	±1.95	1,733	±2.40
	14	1,191	±1.92	2,107	±2.22
	16	1,387	±2.32	2,572	±2.05

COVERED PLOTS

A study was made of the yields from covered plots. Except for the fact that covers were placed over the plots to prevent additional moisture from falling on them, the treatments in these experiments were similar to those employed in the study of border effect on open plots. For a detailed description of the covers, see Kezer and Robertson.[3] The plots received only 7 inches of water from the time of planting to harvest. These experiments were conducted from 1929 to 1931. In 1929 and 1930 six replications were used, and in 1931 three. In diking the plots to facilitate irrigation, the outside rows were more or less covered. The effect of the partially developed outside rows on the middle border rows under cover was determined by correlating the grain yield of row 1 plus row 16 and row 2 plus row 15.

The following correlations were found: -0.2548 ± 0.0997 in 1929; $+0.0109 \pm 0.0938$ in 1930; $+0.3234 \pm 0.1036$ in 1931. All of the correlations were small and indicate very little relationship between the yield of the outside and middle border rows. In determining the correlations only 18 plots were used. In discussing the border effect of the covered plots, rows 1 and 16 are not used, since only fragmentary stands in these rows were obtained.

The data from the covered plots are given in table 6. The effect of eliminating the influence of rainfall by covering the plots during rainstorms and at night is shown by the slightly increased yield ratio in the plots irrigated at jointing. The border effect remains the same for 12- or 14-row plots in different years. These results are similar to those obtained with the uncovered plots. The straw yield and total yield of grain and straw show similar results.

A comparison of the percentage increase of the 12- and 14-row plots over the 10 center rows (table 6) indicates a slightly greater increase in yield of grain in 1931 for the 12- and 14-row plots. However, the increase was similar for all treatments. These results again indicate that there is no difference in comparative yields of the different-sized plots for the different treatments. The same is true for the straw yield.

TABLE 6.—*Comparison of yield of 10 center rows and of 10 center rows plus 2 or 4 border rows, covered plots, 1929–31*

Year	Rows	Grain yield					
		Fall		Germination		Jointing	
	Number	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
1929	10	501	100	438	100	564	100
	12	621	124	539	123	690	122
	14	797	159	698	159	885	157
1930	10	386	100	399	100	464	100
	12	490	127	500	125	587	127
	14	651	169	667	167	795	171
1931	10	147	100	183	100	202	100
	12	196	133	240	131	266	131
	14	271	184	317	173	325	161
Average	10	345	100	340	100	410	100
	12	436	126	426	125	514	125
	14	573	166	561	165	668	163

[3] KEZER, A., and ROBERTSON, D. W. See footnote 2; pp. 80–116.

The probable error is greater for the covered plots (table 7) than for the open plots. In 2 years out of 3 the error decreased as the number of rows increased. In 1929 both the grain-yield and the straw-yield error was smallest for the 10-row plot. The probable error, however, varied more between years than it did between plots. These data indicate that Marquis wheat receiving irrigations at different times gives comparable yields as accurately from plots consisting of 10 center rows plus 4 border rows as from the 10 center rows alone.

TABLE 7.—*Probable error in percentage of mean for plots of 10 rows, 10 rows plus 2 border rows, and 10 rows plus 4 border rows; covered plots, irrigated in the fall, 1929–31*

Year	Rows	Grain yield	Probable error	Straw yield	Probable error
	Number	*Grams*	*Percent*	*Grams*	*Percent*
1929	10	501	±2.44	1,037	±1.52
	12	621	±2.51	1,277	±1.71
	14	797	±2.47	1,610	±1.76
1930	10	386	±4.56	860	±2.89
	12	490	±4.29	1,091	±2.76
	14	651	±3.76	1,455	±2.54
1931	10	147	±7.69	448	±2.90
	12	196	±6.78	598	±2.49
	14	271	±5.20	724	±2.30

STATISTICAL ANALYSES

A summary of the yields in relation to their probable errors is given in table 8.

TABLE 8.—*Total and average yields of grain from covered plots consisting of 10 center rows plus 0, 2, 4, and 6 border rows*

Year	Time of irrigation	Data for yield in grams per plot of number of rows indicated							
		10 rows		12 rows		14 rows		16 rows	
			D/PE		D/PE		D/PE		D/PE
1928	In fall	1,384±38.00	.....	1,647±41.00	.....	2,023±45.00	.....	2,551± 73.00	.....
	At germination	1,527±42.00	.....	1,839±46.00	.....	2,238±50.00	.....	2,900± 83.00	.....
	At jointing	1,533±42.00	.....	1,815±45.00	.....	2,229±49.00	.....	2,870± 82.00	.....
	Difference:								
	Germination-fall	143±56.64	2.52	192±61.62	3.12	215±67.27	3.20	349±110.53	3.16
	Jointing-fall	149±56.64	2.63	168±60.87	2.76	206±66.53	3.10	319±109.77	2.91
1929	In fall	1,020±23.00	.....	1,226±27.00	.....	1,535±28.00	.....	2,007± 32.00	.....
	At germination	884±20.00	.....	1,090±24.00	.....	1,383±25.00	.....	1,831± 29.00	.....
	At jointing	1,434±33.00	.....	1,715±38.00	.....	2,114±38.00	.....	2,707± 43.00	.....
	Difference:								
	Germination-fall	−136±30.48	4.46	−136±36.12	3.77	−152±37.54	4.05	−176± 43.19	4.07
	Jointing-fall	414±40.22	10.29	489±46.62	10.49	579±47.20	12.26	700± 53.60	13.06
1930	In fall	1,180±27.00	.....	1,389±30.00	.....	1,718±32.00	.....	2,210± 28.00	.....
	At germination	1,144±26.00	.....	1,350±29.00	.....	1,618±30.00	.....	2,006± 25.00	.....
	At jointing	1,390±31.00	.....	1,674±36.00	.....	2,063±39.00	.....	2,555± 32.00	.....
	Difference:								
	Germination-fall	−36±37.50	.96	−39±41.72	.93	−100±43.86	2.28	−204± 37.54	5.43
	Jointing-fall	210±41.11	5.11	255±46.86	6.08	345±50.45	6.84	345± 42.52	8.11
1931	In fall	825±14.00	.....	994±19.00	.....	1,191±23.00	.....	1,387± 32.00	.....
	At germination	757±13.00	.....	910±18.00	.....	1,097±21.00	.....	1,336± 31.00	.....
	At jointing	1,111±18.00	.....	1,348±26.00	.....	1,596±31.00	.....	1,861± 43.00	.....
	Difference:								
	Germination-fall	−68±19.10	3.56	−84±26.17	3.21	−94±31.14	3.02	−51± 44.55	1.14
	Jointing-fall	286±22.80	12.54	354±32.20	10.99	405±38.60	10.49	474± 53.60	8.84
Average	In fall	1,102±12.00	.....	1,314±15.00	.....	1,617±16.00	.....	2,039± 21.00	.....
	At germination	1,078±12.00	.....	1,297±14.00	.....	1,584±15.00	.....	2,018± 21.00	.....
	At jointing	1,367±15.00	.....	1,638±18.00	.....	2,001±19.00	.....	2,498± 26.00	.....
	Difference:								
	Germination-fall	−24±16.97	1.41	−17±20.52	.83	−33±21.93	1.50	−21± 29.70	.71
	Jointing-fall	265±19.21	13.79	324±23.43	13.83	384±24.84	15.46	459± 33.42	13.73

The difference between the plots irrigated in the fall and those irrigated at germination is not significant, as is shown by the D/PE for the average yields. The degree of significance varies, however, in different years. For 1928 the deviation divided by the probable error of a difference is more than three for plots including 2, 4, and 6 border rows. In 1929 all of the differences are significant. In 1930 only the 16-row plots (10 center rows + 6 border rows) show a significant difference. In 1931 the 10-, 12-, and 14-row plots show a significant difference.

When the difference between the plots irrigated at jointing and those irrigated in the fall are considered, a significant difference is found only for the 14-row plot in 1928. However, in all of the other years there is a significant difference for all of the plots. There is no consistent increase, however. In 1928 there is a slight increase in the size of the difference divided by the probable error of a difference up to 14 rows, then there is a slight decrease. In 1929 and 1930 there is an increase up to the 16-row plots, but in 1931 there is a decrease, the 10-row plots having the greatest difference. The data for the average yields show no general trend in the size of the difference. The average results when interpreted in relation to their probable errors show a significant difference between the plots irrigated in the fall and those irrigated at jointing for all combinations of the center 10 rows with the various number of border rows. The difference between the fall-irrigated plots and the plots irrigated at germination is not significant in any of the comparisons made on average yields. These results again indicate that comparable results can be obtained from irrigated plots whether the border is removed or whether it is included in the yields.

Similar results were obtained with the covered plots.

SUMMARY

The effect of different numbers of border rows on the yield of plots of Marquis wheat receiving different irrigation treatments was studied.

The yield increased as the size of the plot increased, but the percentage increase was uniform for the three different treatments employed.

Although the average yearly yields varied, the border effect remained uniform with the exception of the outside rows (1 and 16). In 1931, which was a very dry year, less border effect was found from the outside rows.

The variation as determined by the percentage probable error did not increase or decrease for plots of different sizes. It varied in different years with the different number of border rows. The variation was similar for both grain and straw yields.

When the same amount of irrigation water was applied to Marquis wheat at different times, the comparable yields were the same for plots of 10 rows, and for 10 plus 2, 4, or 6 border rows.

The difference in the average yield between the plots irrigated in the fall and those irrigated at germination was not significant for plots of 10, 12, 14, or 16 rows; the difference in the average yield between plots irrigated in the fall and those irrigated at jointing was significant for plots of 10, 12, 14, or 16 rows.

THE INFLUENCE OF PHOSPHORUS DEFICIENCY IN DAIRY COWS ON THE COEFFICIENT OF DIGESTIBILITY AND THE BALANCE OF CALCIUM AND PHOSPHORUS[1]

By W. H. RIDDELL, *dairy production investigations, Department of Dairy Husbandry;* J. S. HUGHES, *animal nutrition, Department of Chemistry;* and J. B. FITCH, *head of Department of Dairy Husbandry, Kansas Agricultural Experiment Station*

INTRODUCTION

A frequent observation in cases of aphosphorosis in cattle is the general lack of thrift and the poor condition of the animals affected.[2] Theiler and his associates,[3] after extensive investigation of the problem in South Africa, concluded that phosphorus was a limiting factor in growth and voluntary food consumption and a dominating factor in the maintenance of live weight under ordinary conditions of veld grazing.

In a study of the energy requirement per pound of gain for dairy cows on a low phosphorus ration, Eckles and Gullickson [4] reported that a plane of feeding at least 120 percent of that specified by the feeding standards in general use was necessary to even maintain the live weight of the experimental animals.

It has been definitely established that there is a lowered feed utilization in the phosphorus-deficient animal. The question may be raised, therefore, as to whether the condition of aphosphorosis adversely affects the digestibility of the ration. The evidence bearing on the effect of a lowered mineral intake on digestion is limited. Evans [5] reported the results of a series of digestion trials with swine suffering from prolonged calcium deficiency. Twenty-two trials showed "no enhanced effect on the digestibility of the organic constituents of the food on adding calcium carbonate to a lime-deficient ration." Woodman and Evans [6], working with sheep, concluded that a deficiency of calcium and phosphorus in forage did not cause it to be digested any less efficiently.

These results would suggest that the prolonged feeding of a phosphorus-deficient ration in all probability would not materially influence digestion. However, since there appeared to be no published results of digestion trials for animals in advanced stages of aphosphorosis, it was planned to secure definite information on this subject and on the mineral metabolism of affected animals at the same time.

EXPERIMENTAL PROCEDURE

Late in October 1929, three animals were selected and placed on the low-phosphorus experimental ration: (1) Control cow, E-7 (re-

[1] Received for publication July 24, 1933; issued March 1934. This paper represents a portion of a thesis presented by W. H. Riddell in partial fulfillment of the requirements for the degree of doctor of philosophy, University of Minnesota, 1932. Contribution no. 88 of the Department of Dairy Husbandry, and no. 174 of the Department of Chemistry.

[2] An excellent and comprehensive review of the literature pertaining to phosphorus deficiency in ruminants has appeared in the following publication: THEILER, A., and GREEN, H. H. APHOSPHOROSIS IN RUMINANTS. Nutrition Abs. & Rev. 1: 359–385. 1932.

[3] THEILER, A., GREEN, H. H., and DU TOIT, P. J. PHOSPHORUS IN THE LIVESTOCK INDUSTRY. Union So. Africa Dept. Agr. Jour. 8: 460–504, illus. 1924.

[4] ECKLES, C. H., and GULLICKSON, T. W. THE RELATION OF PHOSPHORUS DEFICIENCY TO THE UTILIZATION OF FEED. Amer. Soc. Anim. Prod. Proc. 1927: 16–21. 1928.

[5] EVANS, R. E. THE INFLUENCE OF THE ADDITION OF CALCIUM CARBONATE TO A RATION LOW IN LIME ON THE APPETITE AND DIGESTIBILITY OF THE FOOD IN SWINE. Jour. Agr. Sci. [England] 19: [799]–801. 1929.

[6] WOODMAN, H. E., and EVANS, R. E. NUTRITIVE VALUE OF PASTURE. VI. THE UTILIZATION BY SHEEP OF MINERAL-DEFICIENT HERBAGE. Jour. Agr. Sci. [England] 20: [587]–615. 1930.

ceiving phosphorus supplement), a 3-year-old, weight 885 pounds, freshened September 14, 1929; (2) low-phosphorus cow, E-8, a 2-year-old, weight 845 pounds, freshened September 26, 1929; (3) low-phosphorus cow, E-9, a 2-year-old, weight 834 pounds, freshened October 7, 1929. All were good milkers, producing between 30 and 40 pounds of milk daily.

The animals were maintained as far as possible on a uniform plane of nutrition, which was 110 percent of the estimated digestible nutrients required as prescribed by the Morrison feeding standard. This intake was adjusted at the beginning of each month to meet changes in the milk production and live weight of the animals involved or as the circumstances of the experiment called for.

The typical ration furnished at the outset of the experiment was as follows: Prairie hay, 10 pounds; beet pulp (dry), 7 pounds; molasses, 3 pounds; blood flour, 1.6 pounds; grain mix (corn and oats), 3 pounds. This ration contained approximately 0.57 percent calcium and 0.12 percent phosphorus. With the phosphorus supplement (100 g monosodium phosphate) added, the phosphorus content was raised to 0.34 percent. While the calcium content of this ration was probably adequate, the phosphorus was present in subnormal amount.

Except during the colder months the cows were in dry lot most of the time when the weather permitted, being turned into the experimental barn only for feeding and milking.

Since the digestion and balance trial was to be run on animals in the phosphorus-deficient condition, the major concern was to induce aphosphorosis in the experimental animals through feeding the low-phosphorus ration. Although there was early evidence of phosphorus deficiency in both cows E-8 and E-9, the trial was not run until the latter part of June 1930. In the interval between the first marked symptoms of aphosphorosis and the period of the trials the low-phosphorus cows had received a phosphorus supplement on two occasions over extended periods, and it was not until the first part of June that they were judged to be again in a suitable condition of phosphorus deficit. Proof of this was furnished by the symptoms of depraved appetite, low efficiency of feed utilization, and also by the blood analysis. At the conclusion of the trial the inorganic phosphorus content of the blood of the experimental animals was as follows: Cow E-7 (control), 8.72 mg per 100 cc of whole blood; cow E-8 (low P.), 1.34 mg; cow E-9 (low P.), 1.2 mg.

Since the low-phosphorus cows would not consume the entire ration at all times because of loss of appetite, it was decided that during the 10-day preliminary period and the actual trial all 3 cows should be limited to an amount of feed which previous experience had indicated that they would completely consume. In this way the troublesome problem of refused feed was avoided. The amounts fed were as follows: Prairie hay, 4 pounds; beet pulp (dry), 4 pounds; molasses, 2 pounds; blood flour, 1.2 pounds; grain mix (corn and oats), 7 pounds. A sufficient supply of the several feeds was set aside at the outset of the digestion trial under such conditions that no significant change in moisture content would occur during the 10-day feeding period.

Complete collection of the excreta and milk was made. Toluene was used as a preservative in the collection of feces and urine. At the end of each 24-hour collection period an aliquot of the feces was taken

and placed immediately in a drying oven. Aliquot samples of urine and milk were taken daily, preserved with toluene and formaldehyde, respectively, and kept in an ice box. The necessary chemical analyses for the calculation of digestibility of dry matter, crude protein, crude fiber, and the balance of calcium and phosphorus were made.[7]

PRESENTATION AND DISCUSSION OF DATA

The amounts of the different nutrients consumed, voided, and apparently digested are shown in table 1.

TABLE 1.—*Amounts of the different nutrients consumed, voided, and apparently digested by each cow during the trial*

Feed or cow no.	Dry matter	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
NUTRIENTS CONSUMED (GRAMS)					
Prairie hay	16,596	772	5,599	8,423	548
Beet pulp	16,725	1,476	3,345	10,771	105
Molasses	6,798	341		5,644	36
Blood flour	4,952	4,590	91	7	53
Corn and oats	28,716	3,397	950	22,564	1,330
Total	73,787	10,576	9,985	47,409	1,982
NUTRIENTS VOIDED IN FECES (GRAMS)					
E-7 (control)	22,440	5,015	4,066	9,774	549
E-8 (low P.)	20,660	4,884	3,772	9,245	465
E-9 (low P.)	23,320	5,087	4,533	10,469	554
NUTRIENTS APPARENTLY DIGESTED (GRAMS)					
E-7 (control)	51,348	5,561	5,919	37,634	1,433
E-8 (low P.)	53,128	5,692	6,263	38,163	1,517
E-9 (low P.)	50,468	5,489	5,452	36,940	1,429
COEFFICIENTS OF DIGESTIBILITY (PERCENT)					
E-7 (control)	69.6	52.6	59.3	79.4	72.3
E-8 (low P.)	72.0	53.8	62.7	80.5	76.5
E-9 (low P.)	68.4	51.9	54.6	77.9	72.1
Average for low-P. cows	70.2	52.8	58.6	79.2	74.3

The digestion coefficients indicate that the ration employed in the trial was of average digestibility. The observed differences between the low-phosphorus animals and the control are not sufficiently marked or consistent to be regarded as significant. This reasonably close agreement between the coefficients of digestibility for the three animals supports the conclusion that phosphorus deficiency does not lower the animal's ability to digest its feed even when it is in an advanced stage of the disorder. From this it is evident that inefficient feed utilization in the phosphorus-deficient animal is not caused by any inability on the part of the animal to digest its feed properly. When the fact is considered that phosphorus is required in relatively small amounts in the processes of digestion, there is probably little reason why a shortage of this element in the ration should depress digestibility of the feed. Any deficiency in the ration is made up, undoubtedly, from the available reserves of the animal.

The data relating to the calcium and phosphorus balances of the experimental cows are given in table 2.

[7] The analyses were made under the direction of Prof. W. L. Latshaw, of the chemistry department.

TABLE 2.—*Average daily calcium and phosphorus balances of experimental animals during digestion period*

CALCIUM

Cow no.	Feces	Urine	Milk	Outgo	Intake	Balance
E-7 (control)	26.9	0.4	7.8	35.1	41.5	+6.4
E-8 (low P.)	27.1	5.0	9.7	41.8	41.5	−.3
E-9 (low P.)	30.1	4.0	8.9	43.0	41.7	−1.3

PHOSPHORUS

E-7 (control)	25.5	1.3	7.6	34.4	39.6	+5.2
E-8 (low P.)	10.3	.1	8.5	18.9	14.9	−4.0
E-9 (low P.)	10.2	.1	8.1	18.4	14.9	−3.5

A consideration of the mineral-balance data brings out quite clearly the influence of the level of phosphorus intake on the calcium and phosphorus metabolism in the experimental animals. While cows E-8 and E-9 on a phosphorus intake of 14.9 g daily, as contrasted with 39.6 g for the control, showed negative daily balances of 4 g and 3.5 g, respectively, a positive balance of 5.2 g daily was recorded for E-7. It may be concluded, therefore, that the basal ration, while falling considerably short of phosphorus requirements, proved adequate and allowed for some storage when supplemented with 100 g of sodium phosphate daily.

It is interesting to note the uniformity of results for the different paths of outgo in the low-phosphorus cows. Apparently depletion of mineral reserves had reached approximately the same stage for each of these cows at the time of the trial. Attention is also directed to the greater urinary excretion of phosphorus in the case of cow E-7. The urine of the low-phosphorus cows was extremely low in this mineral, containing about one tenth that of the control. On the other hand, in the case of calcium just the reverse was true, namely, the urine of the control contained only approximately one tenth as much calcium as did the urine of the deficient animals.

The close association of calcium and phosphorus in metabolism is well illustrated in the data on the calcium and phosphorus balances. It will be observed that the negative phosphorus balances in cows E-8 and E-9 were accompanied by calcium balances of similar character, though not so marked in extent. On the other hand, the control cow, E-7, showed considerable storage of these minerals during the trial. It is evident that the heavy demand for phosphorus in the deficient animals must have resulted in a considerable break-down of skeletal material.

SUMMARY

The results of a study of the problem of lowered feed utilization in phosphorus-deficient cattle are reported.

Lactating dairy cows in advanced stages of aphosphorosis were found to digest their feed as efficiently as the normal control.

The lowered feed utilization by the phosphorus-deficient cattle did not result from inefficient digestion of the feed. Cows in this condition were found to be in negative balance for both calcium and phosphorus.

A STUDY OF THE IODINE CONTENT OF PENNSYLVANIA POTATOES[1]

By DONALD E. H. FREAR

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INTRODUCTION

This paper reports the iodine content of 135 samples of potatoes, of the 1931 crop, from 135 localities representing 62 of the 67 counties of the State of Pennsylvania. The possible relations of such iodine determinations to the soil types, fertilizer applications, variety and size of potatoes, and incidence of goiter on the farms on which the potatoes were grown and among the students of the Pennsylvania State College, are also pointed out.

The potato was chosen as the subject of this study because it probably forms a larger part of the diet of the people of the State than does any other single food except bread, because it is grown in all sections of the State, and because it is largely consumed in the locality in which it is produced.

The findings in such a study have greater significance for rural than for urban dwellers, as the former are much more likely than the latter to use locally produced potatoes as well as other foods, and the iodine content of potatoes is probably indicative—to some extent—of the iodine content of other foods and feeding stuffs grown in these localities.

The fact that deficient intake of iodine is the predominant cause of simple nutritional goiter has been thoroughly established through extensive surveys and experiments; but inasmuch as acute or chronic focal infection and generalized infection, such as influenza, may play a prominent secondary, or a relatively unimportant primary role of activation, in the production of thyroid disease (*3; 7; 8; 17, p. 135; 23; 24*),[3] it is not to be expected that the iodine content of the diet will be found, under all conditions, in significant correlation with the incidence of goiter; and much less is it to be expected that the iodine content of a single food will be found to be so correlated.

The 135 iodine determinations reported, therefore, are presented mainly as indicating the value of potatoes from Pennsylvania as a source of iodine. There is some (though comparatively little) evidence of iodine deficiency in foods for human beings and livestock in that State.

[1] Received for publication Sept. 27, 1933; issued March, 1934. Technical Paper 604, Agricultural Experiment Station of Pennsylvania.

[2] The author is greatly indebted to the county agricultural agents, and to the farmers who supplied the potatoes. The author is also indebted to Dr. W. B. Mack and E. P. Brasher for assistance in designing the apparatus; to O. J. Kahlenberg for cooperation in the iodine determinations; to Profs. F. G. Merkle and F. J. Holben for advice concerning soil types, and to Drs. J. P. Ritenour and C. D. Dietterich for the data on the medical examinations of students.

[3] Reference is made by number (italic) to Literature Cited, p. 181.

METHOD OF SECURING AND PREPARING SAMPLES

In April 1931 each county agricultural agent in Pennsylvania was asked to name three farmers who would cooperate in furnishing potatoes for iodine analysis. The farms from which these potatoes were to come were to be selected as representative of the predominating types of soil and of farming conditions in each county.

As soon as replies were received from the county agents, a request for 20 average-sized potatoes was sent to each of the farmers named, with a questionnaire calling for information as to the exact location of the farm; the variety of potatoes; the amounts and kinds of commercial fertilizer used to grow the crop; and the incidence of goiter in the farmer's family and among his livestock.

In response to these requests 135 farmers sent potatoes. These samples were weighed in the original condition, and were then thoroughly washed, after which they were sliced and dried at 70° C. The samples were then allowed to come to equilibrium with the air with respect to moisture content, were ground, and enclosed in screw-cap fruit jars. Iodine and hygroscopic moisture were determined in samples weighed in the air-dry condition.

METHOD OF DETERMINATION OF IODINE

After a consideration of McClendon's method, as used by himself and Remington (*14*, *15*, *22*), the open-dish ashing method used by Remington, Culp, and Von Kolnitz (*21*), and the closed-combustion method of Karns (*11*, *12*), the last-mentioned was considered the most accurate, as determined by the recovery of added iodine. It was therefore adopted, in principle, but was modified to obviate the use of solid carbon dioxide and to lessen the cost and the time required in the operation.

The apparatus finally evolved is illustrated in figure 1. The only point of similarity between this apparatus and that of Karns is the combustion flask.[4] The flask used in these determinations was a Pyrex side-neck distilling flask of 2,000 ml capacity, with the side neck cut off to a length of about 6 cm. A Pyrex tube, bent at a right angle, was fused to the middle of the body of the flask, on the side opposite the side neck. This tube had an inside diameter of 8 mm and terminated at a point even with the mouth of the flask. A straight tube of Pyrex glass, of a diameter only slightly smaller than the inside diameter of the neck of the flask, and of such length as to extend from a point 6 cm beyond the end of the neck of the flask to a point 6 cm inside the body of the flask, was placed as indicated, and attached to the neck of the flask with thin-walled rubber tubing. A rubber stopper was fitted into the outer end of the straight tube mentioned; and through this stopper were passed a bent tube of small diameter for the introduction of carbon dioxide and a plunger for progressively raising the sample into the body of the flask as the combustion proceeds.

[4] Mack and Brasher[5] evolved a flask similar to the one described, but with the oxygen inlet as well as the gas outlet fused into the bulb.

[5] BRASHER, E. P. Thesis, Pennsylvania State College. 1932.

During the use of the combustion flask the side neck was connected with a cylinder of oxygen; the tube passing through the rubber stopper was connected with a cylinder of carbon dioxide; and the right-angle tube, attached to the body of the flask, was connected with the manometer and absorption train—as shown in figure 1. The absorption vessels were adaptations of the suggestion of Baumann and Metzger (*1*). These vessels consisted of a Witt filtering apparatus, within the ground-glass joint of which were placed three thicknesses of filter paper (or paper toweling) sufficiently porous to allow the passage of the gases, but serving effectively to condense and to retain the iodine. The two parts of the

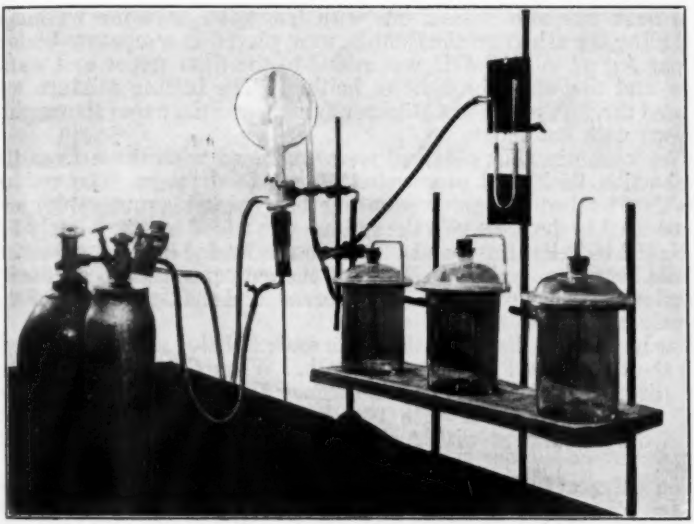

FIGURE 1.—Modified Karns apparatus for the determination of iodine.

vessel were held together with clamps. With the apparatus described, 1 worker, using a duplicate set-up, can make 10 combustions a day without difficulty.

In operation, the combustion flask, oxygen cylinder, and absorption train were connected as shown in the illustration, and a slow stream of oxygen was started into the flask. With the straight Pyrex tube outside the combustion flask, the sample, in a paper tube plugged at both ends with cotton, was introduced into the tube, and raised by means of the plunger until about 1 cm of the length of the paper tube protruded above the end of the Pyrex tube. The cotton at the end of the paper tube was then ignited; the Pyrex tube, carrying the paper tube of sample was quickly inserted in the flask; the rubber tubing which connected the combustion flask and Pyrex tube was then drawn into place, and the combustion was under way. From time to time, as the combustion proceeded, the sample was

elevated by means of the plunger, to expose fresh material for combustion.

If the flame went out, as it sometimes did during the course of a combustion if the supply of oxygen was insufficient, the oxygen was turned off at once, and the carbon dioxide turned on, to extinguish the glowing fire in the sample. This obviated the danger of explosion.

After the last trace of combustible material had been burned, the apparatus was swept out with carbon dioxide. After several minutes of such sweeping, the apparatus was disconnected; the absorption train was washed out with hot water; and the filter paper was cut into strips and placed with the wash water in a beaker. The combustion flask was also washed out with hot water, and the washings, including the ash from the sample, were placed in a separate beaker. About 5 g of solid NaOH was added to the filter paper and washings, and the whole brought to boiling. The boiling mixture was filtered through paper in a Büchner funnel, and the paper thoroughly washed with hot water.

The washings thus obtained were combined with those from the combustion flask, and evaporated almost to dryness. The residue was then washed into an evaporating dish (nickel is preferable) and evaporated to dryness. To the residue was added approximately 5 g of NaOH in pellet form, and the whole was heated over a gas burner. Gentle heat was applied at first, but the temperature was gradually raised until fusion of the NaOH occurred, and the destruction of the organic matter was accomplished.

The melt in the dish was allowed to cool; distilled water was added, and the dish placed on the steam bath. When the contained solids had disintegrated to a pasty consistency the whole was allowed to cool and was extracted with 95-percent ethyl alcohol, previously purified by treatment with $AgNO_3$ and KOH, and distillation over KOH.

The extract (volume about 40 ml) was evaporated, and if the residue contained any organic matter this was fused again with an excess of NaOH, and extracted as before. The material thus extracted was washed into a 125-ml Erlenmeyer flask, and neutralized with dilute H_2SO_4 to the end point of methyl orange (aqueous solution). One small drop of bromine, purified by the method of Karns (*13*), was then added, together with a glass bead, and the mixture shaken until a distinct orange color developed. The bromine was then boiled off, and the solution cooled. A small amount of 20-percent solution of KI and 5 drops of starch solution were then added, and the solution titrated with N/1,000 sodium thiosulfate solution, prepared according to the method of Mayr and Kerschbaum (*16*).

DISCUSSION OF METHOD OF ANALYSIS

It is noteworthy that at no point subsequent to the combustion, which took place in a system designed to catch the liberated iodine, were the products containing the iodine exposed to a temperature much higher than the fusion point of NaOH (318° C.). Exposure

to this temperature never occurred except in the presence of a considerable excess of NaOH, a condition which does not permit the volatilization of iodine compounds.

The efficiency of the filter papers (or paper toweling) in collecting the iodine from the gas stream was attested by recovery tests, the results of which are given in table 1.

TABLE 1.—*Recovery of iodine added to potato samples, by the modified Karns method*

Weight of sample (grams)	Iodine present in sample	Iodine added as KI	Iodine recovered	
	Gammas	*Gammas*	*Gammas*	*Percent*
53.3	6.9	50	49.6	99.2
52.1	6.8	60	57.5	95.8
50.9	6.2	100	94.2	94.2
49.4	6.2	100	98.2	98.2
49.0	6.2	100	87.8	87.8
56.1[1]	.45	75.45	69.3	91.3
56.6[1]	.45	75.45	72.9	96.0
Average				94.6

[1] These determinations were made after the completion of those on potatoes; the others were made before the determinations on potatoes had been made.

While the recoveries here recorded are not perfect, they show that only approximately 5 percent of the added iodine was lost. All results reported are the mean of two closely agreeing determinations.

DISCUSSION OF RESULTS

The quantities of iodine found in the potatoes are shown in table 2. For convenience in comparison with other work these results will be discussed on the basis of air-dry material. The maximum quantity of iodine found was 197 parts per U.S. billion, and the minimum was 9 p.p.b. The mean iodine content of all samples, on this basis, was 70.7 p.p.b. On the basis of moisture-free material the maximum iodine found was 216, the minimum was 10, and the mean, 77.8 p.p.b. These values are of about the same magnitude as those reported by Orr (*18*), who found a minimum of 22, and a maximum of 251 p.p.b., for potatoes grown in different parts of England and Scotland. They are considerably lower, however, than the figures reported by Remington, Culp, and Von Kolnitz (*21*), who found that potatoes from South Carolina varied in iodine content between 87 and 554 p.p.b., with an average of 217 p.p.b. in 72 samples. Values for iodine in potatoes compiled by Orr and Leitch (*20*) include analyses by Von Fellenberg (*4*) and by Hercus and Roberts (*10*). These results, together with some analyses made by Orr and Leitch, are on the fresh basis, but assuming 25 percent of dry matter, the values reported range from 16 to 140 p.p.b. Bleyer (*2*), in some analyses reported by Orr and Leitch, found between 80 and 136 p.p.b., and Brasher [6] found between 150 and 240 p.p.b.

[6] BRASHER, E. P. See footnote 5.

TABLE 2.—*Iodine content of potato samples from various Pennsylvania counties*

Sample no.	County of origin	Iodine	
		Air-dry basis	Moisture-free basis
		P.p.b.	*P.p.b.*
1	Centre	46	50
2	Crawford	61	67
3	Elk	46	51
4	Perry	101	113
5	Cameron	97	106
6	Mercer	116	129
7	York	139	149
8	Juniata	138	158
9	Bradford	99	110
10	Luzerne	109	123
11	Lancaster	122	133
12	Snyder	56	62
13	Indiana	134	148
14	Somerset	83	89
15	Monroe	103	113
16	Indiana	197	216
17	Cumberland	61	67
18	Lackawanna	49	55
19	Luzerne	148	163
20	Warren	99	109
21	Schuylkill	106	118
22	Bedford	86	94
23	Wayne	60	65
24	Mifflin	116	125
25	Wayne	48	52
26	Dauphin		
27	Cameron	32	35
28	Clearfield	35	38
29	Allegheney	20	22
30	Fayette	66	75
31	Bucks	73	80
32	Union	40	44
33	Northumberland	66	73
34	Huntingdon	155	167
35	Venango	169	192
36	Northampton	63	68
37	Perry	73	79
38	Dauphin	85	94
39	Northumberland	58	65
40	Mifflin	26	29
41	Philadelphia	162	178
42	Delaware	74	80
43	York	33	37
44	Lebanon	83	91
45	Delaware	122	135
46	Philadelphia	77	86
47	Lancaster	45	50
48	Lycoming	17	18
49	Warren	92	100
50	Elk	27	30
51	Juniata	61	68
52	Erie	140	151
53	Mifflin	11	12
54	Blair	148	161
55	Lawrence	92	102
56	Potter	45	50
57	Bucks	39	42
58	Venango	75	81
59	Erie	167	185
60	Berks	60	65
61	Lebanon	47	53
62	Perry	43	46
63	Lycoming	85	92
64	Lackawanna	100	107
65	McKean	71	77
66	Bucks	126	138
67	Westmoreland	60	66
68	Clinton	26	29
69	Somerset	48	54
70	Clinton	9	10
71	Pike	90	103
72	Lehigh	66	74
73	do	101	113
74	Snyder	59	64
75	Beaver	111	125
76	Lycoming	66	72
77	Forest	22	24
78	Cambria	119	132
79	Butler	32	35
80	Forest	91	101
81	Erie	77	87
82	Venango	71	80
83	Union	91	101
84	Butler	95	105
85	Clearfield	40	44
86	Potter	50	55
87	Carbon	10	11
88	McKean	43	47
89	Luzerne	67	76
90	McKean	14	15
91	Centre	57	62
92	Crawford	20	22
93	Sullivan	86	94
94	Northampton	99	106
95	Armstrong	56	60
96	Bradford	22	25
97	Clearfield	66	73
98	Bedford	103	111
99	Huntingdon	47	53
100	Adams	58	63
101	Chester	67	73
102	Fayette	26	29
103	Beaver	19	21
104	Bradford	61	66
105	Crawford	101	109
106	Susquehanna	70	76
107	Clarion	54	59
108	Schuylkill	61	65
109	Mercer	82	88
110	Elk	123	134
111	Wyoming	53	58
112	Berks	39	42
113	Beaver	44	48
114	Adams	58	63
115	Northumberland	55	61
116	Pike	127	136
117	Blair	76	82
118	Northampton	26	29
119	Cambria	29	32
120	Sullivan	53	60
121	Wayne	14	16
122	Cameron	36	40
123	Clinton	39	42
124	Tioga	37	42
125	Greene	18	20
126	Monroe	57	64
127	Chester	38	42
128	Columbia	86	96
129	Armstrong	69	74
130	Franklin	19	21
131	Wyoming	24	27
132	Franklin	112	126
133	Allegheney	155	166
134	do	57	61
135	Blair	21	23
136	Franklin	48	52

RELATION OF IODINE CONTENT OF POTATOES TO GEOGRAPHICAL DISTRIBUTION

For the consideration of the iodine content of the potatoes as related to the locality of origin the iodine values were grouped and averaged to represent six arbitrary divisions of the State, as indicated in figure 2. Potatoes from the north-central part contained significantly less iodine than did potatoes from the other sections. The mean difference between the iodine content of potatoes grown in the north-central and those grown in the southeastern division was 31 ± 8 p.p.b., a figure which may be considered significant, since the odds against such a difference occurring by chance alone are 100 to 1. The differences between the iodine content of the potatoes from the north-central section and those grown in the other four divisions of the State are not so significant, in the light of such statistical analysis, even though as in the case of the northwestern section, the numerical difference is greater.

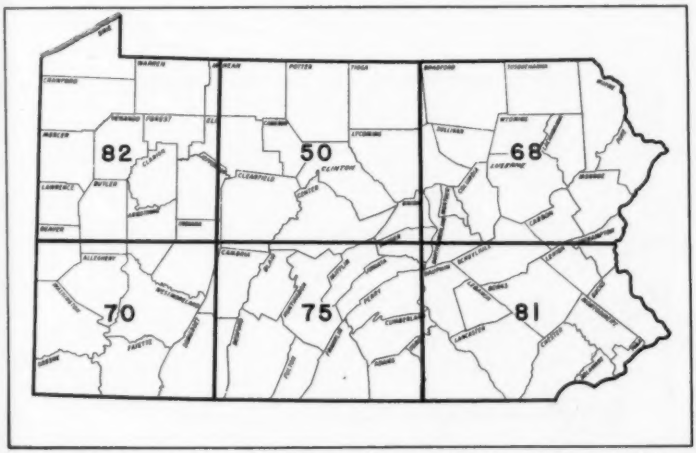

FIGURE 2.—Relation of iodine content in parts per billion of samples of potatoes to geographical origin.

RELATION OF IODINE CONTENT OF POTATOES TO SOIL TYPE

The potato samples were divided into groups according to the soil types on which they were grown. There are two predominant soil types in Pennsylvania, a nonglaciated, residual soil, derived from sandstones and shales, which comprises about 62 percent of the area of the State and extends over the central and southwestern portions; and glaciated soils of similar origin found in the northwestern and northeastern sections of the State, and covering about 24 percent of the total area.

A third type of soil is derived from calcareous rocks, and lies mainly in the long valleys of the south-central and southeastern sections; a fourth type of soil, derived from igneous rocks, is found

mainly in the southeastern section of the State; and a fifth type is of marine origin, and lies in a narrow strip along the extreme southeastern border of the State.

The average iodine contents of the potatoes from these soil types are shown in table 3. The probable errors of the averages of groups 3, 4, and 5 are presented because of the small numbers of samples.

TABLE 3.—*Iodine content of potato samples grown on various soil types in Pennsylvania*

Soil number and type	Samples	Mean iodine content, air-dry basis
	Number	*P.p.b.*
1. Dekalb and Westmoreland	74	66±4
2. Volusia and associated groups	30	79±6
3. Hagerstown	18	63
4. Penn and Lansdale	6	74
5. Sassafras (marine)	2	142

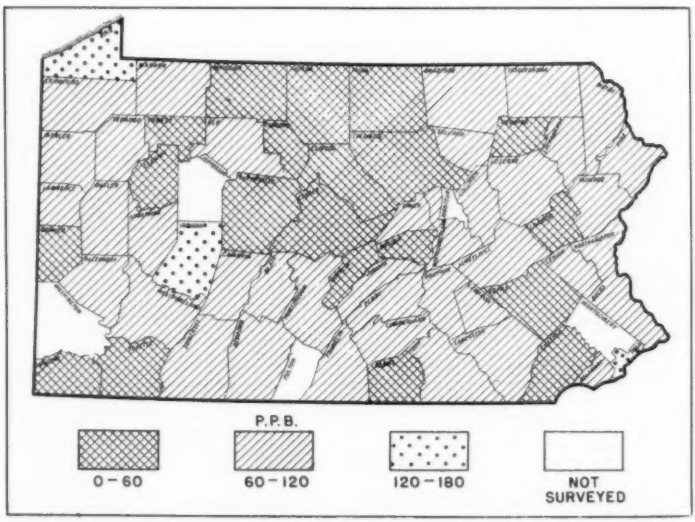

FIGURE 3.—Relation of iodine content of samples of potatoes to their counties of origin.

No significant differences were found between the average iodine contents of the potatoes from the different soil types except that the two samples grown on marine soil were unusually high in iodine.

Figures 3 and 4 represent the distribution of the iodine of the potato samples by counties as compared with a generalized soil map of the State. The potatoes from the north-central area of nonglaciated soil were comparatively low in iodine—as already noted—but as many of the apparently low-iodine counties were outside this area as were within it.

VARIETY OF POTATOES AND IODINE CONTENT

More than 82 percent of the potatoes studied were of some strain of the Russet variety, and about 10 percent were of the Rural New Yorker variety. The average iodine content of those potatoes belonging to the former variety was 72 p.p.b., while the average iodine content of the Rural New Yorker variety was 67.4 p.p.b. This difference is not significant.

RELATION OF SIZE OF POTATOES AND IODINE CONTENT

Since whole potatoes have been shown to contain more iodine than those which have been peeled, it was considered possible that some relation might exist between size (and indirectly the ratio of surface to mass) and the iodine content, but the statistical treatment of the data revealed no such relation.

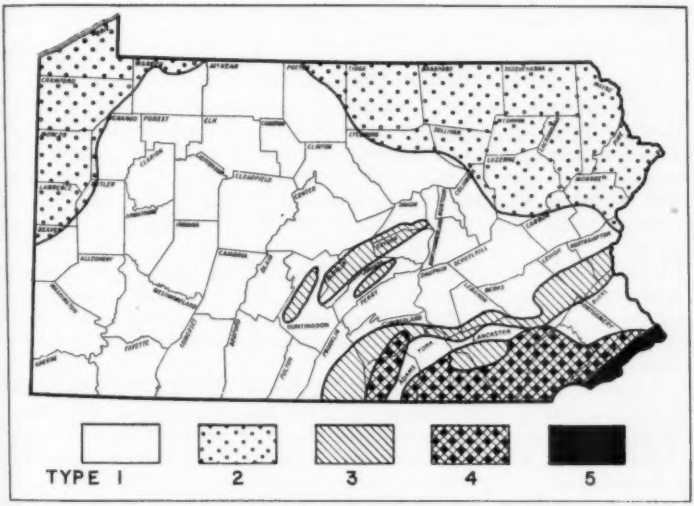

FIGURE 4.—Generalized soil map of Pennsylvania.

RELATION OF IODINE CONTENT OF POTATOES TO FERTILIZER APPLICATIONS

Numerous workers, including Orr, Kelley, and Stuart (*19*), Von Fellenberg (*5*, *6*), Hercus and Roberts (*10*), and Hercus, Benson, and Carter (*9*) have shown that iodine applied to the soil may be taken up by the plant, and that some fertilizer materials, notably Chilean sodium nitrate, contain appreciable quantities of this element. In this study, the samples of potatoes were grouped according to the amounts of nitrogen applied to the crop as commercial fertilizer, but this grouping brought to light no significant relation—as might have been expected in view of the fact that the source of the nitrogen of the fertilizers was not known.

RELATION OF IODINE OF POTATOES TO INCIDENCE OF GOITER

Twenty-four of the 135 farm families which raised the potatoes used in this study reported one or more cases of goiter. Five of these families had 2 cases, there being a total of 29 cases among approximately 675 individuals, or an incidence of 4.3 percent. Attention is called to the fact that these reports were made by the farmers themselves, and minor thyroid enlargements may have passed unnoticed.

Only 6 instances were reported of thyroid derangement among the livestock of the 135 farms.

The iodine content of the potatoes grown on farms on which the farm families had one or more cases of goiter was 87 p.p.b., while the potatoes from the other farms showed an iodine content of 76 p.p.b., both figures being on a moisture-free basis.

RELATION OF IODINE OF POTATOES TO INCIDENCE OF GOITER AMONG FRESHMEN AT THE PENNSYLVANIA STATE COLLEGE

The records of the physical examinations given each of the past five incoming classes at the Pennsylvania State College were examined to determine the incidence of goiter among the 6,354 individuals composing this group. In other studies of this group it has been determined that the rural-urban distribution among these students agrees very closely with such distribution of the population of the State as a whole, as determined by the census figures. Of the 6,354 individuals examined, 5,405 were males and 950 were females.

The frequency of goiter among the student population included in this survey was 2.9 percent. Several other surveys made in past years by various individuals connected with the Pennsylvania Department of Health indicate a state-wide incidence of goiter of between 2 and 3 percent.[7]

In consideration of the slight incidence of goiter in the State, and the uncertain significance of the iodine content of potatoes as indicative of the iodine content of the diet as a whole, no significant correlation was to be expected from this comparison, and none was found.

SUMMARY

One hundred and thirty-five samples of potatoes, grown in as many localities in Pennsylvania, were analyzed for iodine by a modification of the method of Karns. The minimum iodine content was found to be 10 p.p.b., the maximum 216 p.p.b., and the mean 77.8 p.p.b., expressed on a moisture-free basis.

The mean iodine content of the potatoes grown in the southeastern section of the State was found to be significantly higher than that of the potatoes grown in the north-central section, but no other sectional differences in iodine content were significant.

The analyses of potatoes grown on five different soil types in the State showed that those grown on glaciated soils contained slightly more iodine than did those grown on similar nonglaciated

[7] Wood, H. B. Personal communication.

types. Two samples of potatoes grown on marine soil showed a much higher iodine content. The iodine content of the potatoes analyzed was apparently not affected by the fertilization.

No significant correlation was found between the iodine content of the potatoes and the size of the individual potatoes, nor between the iodine content and variety.

Medical examination of 6,354 students at the Pennsylvania State College during the past 5 years showed a goiter incidence of 2.9 percent. No significant correlation was found between the iodine content of the potatoes from the different parts of the State and the incidence of goiter in the same localities, as indicated by the examination of these students.

A similar study of the relation of the iodine content of the potatoes and the incidence of goiter among the 135 farm families showed no significant relation between the two. The goiter incidence in the farm families was 4.3 percent. The potatoes from farms upon which there were one or more cases of goiter showed a considerably higher iodine content than those from farms on which no goiter was reported.

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COMPARATIVE VALUE OF SOME COMMERCIAL PROTEIN SUPPLEMENTS IN THE RATIONS OF GROWING CHICKS [1]

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INTRODUCTION

Among the more important sources of protein concentrates available to the commercial poultrymen in the State of Washington are milk products, trimmings from meat-packing plants, and byproducts from fish canneries. Wide variations have been observed in the results obtained by the use of these different concentrates. The experiment here recorded was conducted to determine the feeding value of these different protein concentrates and blends of these concentrates when used in chick rations recommended by the State College.[2]

EXPERIMENTAL METHODS AND MATERIALS

A group of Single Comb White Leghorn chicks was obtained from commercial hatchery stock. From this group, 9 lots of 20 chicks each were so selected that at the beginning of the trials the chicks in all lots possessed uniform physical characteristics. The lots of chicks were brooded for 8 weeks under the conditions described by St. John, Carver, Helphrey, Miller, and Cassel.[3] The feed consumption and individual chick weights at biweekly periods were recorded and observations were made on the physical condition and mortality of the chicks. The excreta were collected daily and sampled according to the method described by St. John and Johnson.[4]

The all-mash basal ration was made up as follows: 40 pounds ground yellow corn; 20 pounds ground wheat; 10 pounds ground oats; 10 pounds white-wheat bran; 4 pounds dehydrated alfalfa; 1.5 pounds steamed bone meal; 1.5 pounds oyster-shell flour; 0.75 pound salt; and 0.25 pound cod-liver oil. The protein concentrates were adjusted to a 34 percent protein level with cornstarch, so that 12 pounds of the diluted concentrate with 88 pounds of the basal ration gave a protein content of approximately 14 percent for the completed ration.

Previous investigations at this station by Carver, St. John, Aspinall, and Flor [5] have shown that 14 percent of protein is close

[1] Received for publication June 7, 1933; issued March, 1934. Published as Scientific Paper no. 263, College of Agriculture and Experiment Station, State College of Washington.

[2] This work was done cooperatively by the Divisions of Poultry Husbandry and Chemistry.

[3] ST. JOHN, J. L., CARVER, J. S., HELPHREY, J. P., MILLER, W., and CASSEL, L. W. THE EFFECT ON GROWTH OF VARIOUS PROTEIN LEVELS OF DRY SKIM MILK IN A CHICK MASH. Poultry Sci. 9: 320–333, illus. 1930.

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[5] CARVER, J. S., ST. JOHN, J. L., ASPINALL, T. E., and FLOR, I. H. PROTEIN REQUIREMENTS OF CHICKENS. Poultry Sci. 11: 45–57, illus. 1932.

to the lower level of protein consistent with optimum growth, and therefore variations in the value of the various concentrates should bring out differences which would not be apparent if the percentage of protein were closer to the excessive level. Table 1 gives the data on the rations used for each lot of chicks.

TABLE 1.—*Analyses (percent) of the complete ration composed of 88 pounds of basal ration and 12 pounds of the mixture of concentrate and cornstarch*

Lot no.	Protein concentrate	Protein in concentrate	Protein in total ration	Ash in total ration
70	Argentine meat scrap	44.60	14.22	9.10
71	Vico meat scrap	61.70	14.40	6.38
72	Alaska herring meal	73.00	14.30	5.47
73	Fish shreds	59.00	14.20	6.65
74	Skim-milk powder	36.50	14.57	5.93
75	Blend: 4 percent each Argentine meat scrap, herring meal, skim-milk powder		14.10	6.76
76	Blend: 4 percent each Argentine meat scrap, herring meal, skim-milk powder; minerals changed to 1 percent oyster-shell flour; 2 percent cornstarch		14.10	5.24
77	Blend: 4 percent each Argentine meat scrap, herring meal, skim-milk powder; minerals changed to 2 percent oyster-shell flour; 1 percent cornstarch		13.65	5.94
78	Blend: 4 percent each Vico meat scrap, fish shreds, skim-milk powder		14.35	6.23

RESULTS

In considering the efficiency of the various protein concentrates used, the most important criterion from the commercial poultryman's point of view is the actual growth response of the chicks. The

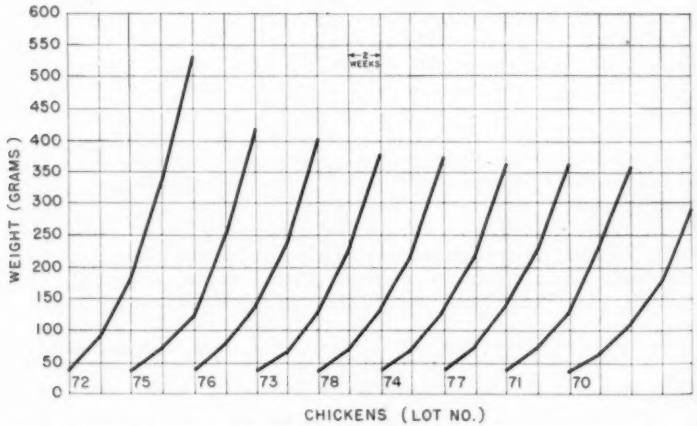

FIGURE 1.—Growth of chicks when fed various protein concentrates (indicated by numbers) for a period of 8 weeks.

growth curves for the 8-week periods are shown in figure 1. To facilitate comparison, these curves are presented in the order of descending value.

The growth of the chicks in lot 72 is strikingly better (525) than that in any of the other lots, while the growth in lot 70 is decidedly

poorer (289). The other lots have a medium value between these two, ranging from 363 to 414 g. Miller and Bearse [6] reported from the Western Washington Experiment Station, a similar trial in which a ration containing 17 percent of protein was used and 14 hours of light, as compared with 14 percent of protein and 12 hours of light in this test. They obtained better growth for all lots. The lower level of protein employed in the present experiment is perhaps responsible for the smaller growth obtained, but this low level of protein would also accentuate any differences in the comparative feeding value of the different protein supplements used. In the general appearance of the birds there were no marked differences between lots other than those to be expected in view of the differences in growth. From the growth response of the birds it would seem that herring meal, the protein supplement used in the ration fed lot 72, was a much better supplement than the other concentrates, and superior particularly to the Argentine meat scrap used in the ration fed lot 70.

The biological values for the protein supplements fed the different lots of chicks were determined by the method described by St. John, Johnson, Carver, and Moore.[7] These values are given in table 2.

TABLE 2.—*Biological values of protein supplements, based on average chick weights for the 2-week periods*

Period	Biological value of protein supplement fed to lot no.—								
	70	71	72	73	74	75	76	77	78
First 2 weeks	62.9	56.3	63.5	59.2	61.4	58.1	62.9	65.4	67.5
2 to 4 weeks	62.7	59.8	61.5	55.2	58.5	65.2	58.8	67.0	70.8
4 to 6 weeks	62.3	55.6	64.4	59.1	65.3	57.6	62.6	65.9	61.0
6 to 8 weeks	71.7	72.1	71.2	63.0	64.9	69.1	63.2	70.0	70.8
Average	64.9	61.0	65.1	59.1	62.5	62.5	61.9	67.1	67.5

There does not appear to be any significant relation between the actual percentage of protein retained by the chicks and their growth. When the gain in weight per gram of protein ingested is considered, however, there appear variations which are significant. The gains in weight per gram of protein ingested are given in table 3.

The chicks in lot 72 made the greatest gain in weight per gram of protein for the first 6 weeks. The biological values of lot 72 did not differ greatly from those of lot 70, which made the lowest gain per gram of protein and also showed the poorest growth response. The chicks in all lots had free access to an unlimited feed supply, since it was desired to study their reaction under conditions as nearly comparable to those in the field as would be consistent with controlled experimental procedure. The amount of feed consumed by the chicks varied greatly between lots and the variations in growth of the chicks in the different lots may be explained on this basis.

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TABLE 3.—*Gain in chick weight (grams) per gram of protein consumed, and average weight (grams) of feed consumed per chick, when various protein supplements were fed*

GAIN IN WEIGHT

Period	Lot 70	Lot 71	Lot 72	Lot 73	Lot 74	Lot 75	Lot 76	Lot 77	Lot 78
First 2 weeks	2.02	2.43	2.66	2.34	2.34	2.48	2.63	2.32	2.37
2 to 4 weeks	1.75	1.82	2.34	2.12	1.96	2.28	2.03	1.86	1.84
4 to 6 weeks	1.86	2.11	2.48	2.20	1.97	2.09	2.26	1.72	1.82
6 to 8 weeks	1.31	1.64	1.78	1.74	1.75	1.76	1.97	1.73	1.79
Average	1.70	1.87	2.14	2.03	1.92	2.08	2.19	1.81	1.85

FEED CONSUMED PER CHICK

First 2 weeks	103.3	109.9	134.7	102.8	107.5	102.3	111.8	119.8	107.6
2 to 4 weeks	178.4	211.4	265.8	193.6	201.4	215.4	210.4	245.2	211.2
4 to 6 weeks	283.2	330.1	421.9	319.1	296.8	361.7	307.3	363.6	343.2
6 to 8 weeks	552.1	565.5	780.0	602.7	598.6	663.6	596.2	608.4	612.6
Average	1,104.0	1,214.9	1,596.2	1,179.8	1,185.8	1,333.1	1,184.8	1,337.0	1,274.6

The birds in lot 72 consumed more feed and made greater gains per gram of protein ingested than those of any of the other groups. The amount of feed consumed by lot 70 while the lowest for all the groups, was not sufficiently low to account for the poor growth of this pen. In the case of lot 70, poor growth may have been due to the high ash content of the ration. Frasier and Annonen,[8] using the same kind of meat scrap that was employed in this study, found evidence of a close association between high-ash content and low chick weight. As the analyses in table 1 show, lot 70 is the only lot to which this conclusion can be applied. Factors other than protein that might have influenced the results of this study are now being investigated.

SUMMARY

A study was made to determine the value of various protein concentrates used as supplements to chick rations. The protein in the ration was maintained at approximately 14 percent for all lots of chicks.

The chicks fed Alaska herring meal made the best growth, those fed Argentine meat scrap made the poorest, while those fed the concentrates and blends of concentrates showed a growth about midway between the two.

The biological values were determined for each lot of chicks, but could not be associated with other data to show that the better growth in some lots was due to a better utilization of the protein in the ration.

While the lot showing the greatest gain in weight per gram of protein ingested made the best growth, this association did not hold for the other lots. The lot making the greatest gain per gram of protein also showed the greatest feed consumption.

[8] FRASIER, F. W., and ANNONEN, W. BIOLOGICAL VALUES OF FISH MEALS AND MEAT MEALS. Unpublished thesis. State College of Washington. 1932.

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